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Ecosystem properties of acid grasslands along a gradient of nitrogen deposition

**Thesis submitted for the degree of PhD
on the 30th September 2004**

Carly Stevens BSc (Hons) MSc

**Department of Earth Sciences and Department of Biological Sciences
The Open University**

**NERC Centre for Ecology and Hydrology
Monks Wood**

Abstract

Sixty-eight randomly selected grasslands belonging to the National Vegetation Classification group U4 (*Festuca ovina*-*Agrostis capillaris*-*Galium saxatile* grassland) were studied during the summers of 2002 and 2003 along a gradient of atmospheric N deposition ranging from 6 to 36 kg N ha⁻¹ yr⁻¹ to investigate potential damage caused by acidification and eutrophication. At each site vegetation was surveyed and samples were taken from the topsoil and subsoil. Above-ground plant material was collected from three species: *Agrostis capillaris*, *Galium saxatile* and *Rhytidadelphus squarrosus*.

Species richness showed a negative linear trend with N deposition, with a reduction of 1 species for every 2.5 kg N ha⁻¹ yr⁻¹. At the current mean N deposition in Europe this gave a reduction in species richness of 23% from a projected pristine condition. Forbs accounted for the majority of this decline, showing reductions in both cover and abundance along the N-deposition gradient. Grasses showed a decrease in species richness, but an increase in cover, indicating increased dominance of a few species. Using canonical correspondence analysis, several individual species were identified as being positively and negatively associated with N deposition.

Soil pH declined along the N-deposition gradient. This was also associated with increased mobilisation of aluminium, arsenic and lead. Nitrate concentration in the soil did not increase with increasing N inputs, possibly due to rapid plant uptake and its mobility in the soil profile. Soil extractable ammonium concentration showed a

significant positive correlation with N inputs. A weak relationship was also identified between the C:N ratio in the soil and N inputs. Potential ammonium mineralization showed considerable variation across the sites.

There was no correlation between aboveground tissue N and N deposition for any of the three species examined. A weak correlation was identified between tissue N and soil C:N for *G. saxatile*. Tissue N:P ratios gave no clear indication of nutrient limitation.

The relative merits of different indicators of N-deposition are discussed.

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1. Introduction

1.1.1 Nitrogen Pollution

The earth's atmosphere is approximately 80% nitrogen. In its inert form (N_2) it is unavailable for use by most organisms but in its reduced and oxidised forms it is an essential nutrient for plants. However, in these forms it may also be a pollutant. The main nitrogenous air pollutants include nitric oxide (NO), nitrogen dioxide (NO_2) and ammonia (NH_3) which are dry deposited, together with nitrate (NO_3^-) and ammonium (NH_4^+) as wet deposition (Stulen *et al.*, 1998).

Wet deposition occurs when soluble nitrogen compounds are dissolved in rain and cloud drops. Dry deposition consists of particles that are deposited directly to surfaces such as the ground or vegetation. High deposition rates of these nitrogen pollutants are measured in cities, in rural areas close to intensive livestock farms and in upland areas receiving high rates of cloud droplet deposition (Skiba *et al.*, 1998).

Globally, nitrogen deposition is a significant problem causing both acidification (increased acidity of the soils) and eutrophication (a build up of nutrients in the soil). Effects of N deposition have been noted in the heavily industrialised parts of the world including eastern USA, Europe, the former Soviet Union and large parts of Asia. Other areas such as South America and large areas of Africa are also at risk of acidification and eutrophication (Bouwman, 2002). Globally the largest emissions of pollutant nitrogen are in the form of ammonia, emitted from animal excreta, synthetic fertilisers, oceans and biomass burning. Nitrogen oxides account for approximately

1/3 of global emissions (Oliver, 1998). The global nitrogen budget is shown in figure 1.1. The problem of nitrogen deposition is likely to increase as the human population continues to grow (Tilman *et al.*, 2001).

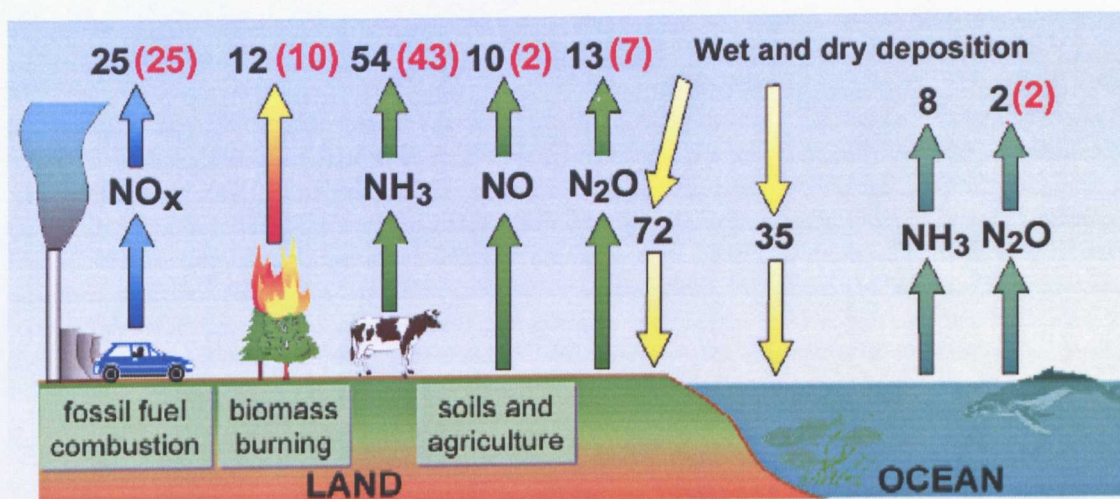


Figure 1.1 Global atmospheric nitrogen budget (Tg N yr^{-1}). Human contributions are shown in brackets (NEGTA, 2001 modified using figures presented by D. Fowler at Global Atmospheric Nitrogen Enrichment 16-18th September, 2003).

Biotic fixation provides about $90\text{--}130 \text{ Tg N yr}^{-1}$ of reactive N. Human activities have resulted in the fixation of an additional $\sim 140 \text{ Tg N yr}^{-1}$ by energy production, $\sim 80 \text{ Tg N yr}^{-1}$ by fertiliser production and $\sim 40 \text{ Tg N yr}^{-1}$ by cultivation of crops (Galloway *et al.*, 1995). The global N cycle has now reached the point where more N is fixed annually by human-driven than by natural processes (Vitousek, 1994). The increasing emission of NO_x (NO , NO_2 , NO_3^-) is a serious issue on a global scale: as industrial development takes place in the Far East it is forecast that global man-made emissions of NO_x will rise from present day levels of $\sim 20 \text{ M tonnes N year}^{-1}$ to $\sim 46 \text{ M tonnes N year}^{-1}$ (RGAR, 1997). Galloway *et al.* (1995) predict that the anthropogenic N-fixation rate will increase by about 60% by the year 2020.

The contribution of ammonia to total nitrogen deposition in the UK varies between 40 and 80 % (Pearson and Stewart, 1993) (see also figure 1.1). Emissions of atmospheric NH_3 from agriculture, primarily from the volatilisation of livestock wastes, represent a loss of nitrogen from the farming system as well as a diffuse environmental pollutant (Sutton *et al.*, 1998). Cattle are responsible for the largest contribution towards ammonia emissions, followed by sheep, poultry and pigs (ApSimon, 1987). High rates of fertiliser manufacture and application are also a major source of emissions, and fermented feeds such as silage may also have a role to play (Pearson and Stewart, 1993). Human activities account for approximately 75% of the mobilisation of reduced N (RGAR, 1997) (figure 1.2).

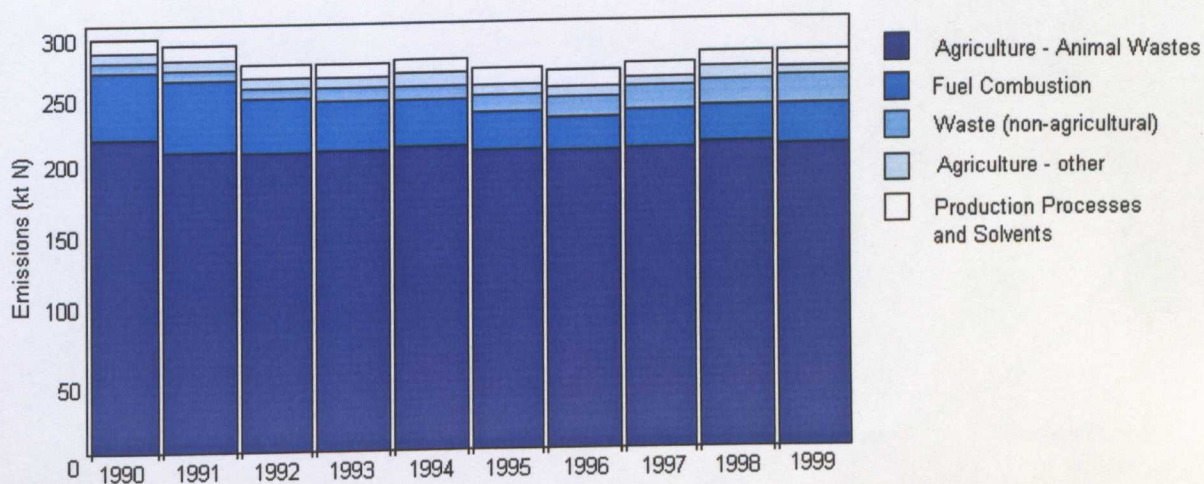


Figure 1.2 Emissions of Ammonia in the UK (kt N) (NEGTA, 2001)

Ammonia deposits very rapidly. It has a lifetime of only a few hours to a few days before it chemically degrades (Oliver *et al.*, 1998). Typical rates of deposition of 15-20 mm s^{-1} were found for short vegetation (Sutton *et al.*, 1993). Dry deposition is rapid, more than 80% is estimated to originate from emissions within a few kilometres. The close integration between agricultural land and semi-natural areas in western Europe leads to a higher deposition of NH_3 in habitats at the margins of

agricultural land. Small, sensitive areas, such as 'island' nature reserves in the agricultural landscape, will receive higher than average deposition (Sutton *et al.*, 1998). Studies have shown that ammonia concentrations are high within the immediate vicinity of livestock buildings, affecting vegetation species composition and foliar nitrogen concentration (Fowler *et al.*, 1998) (Figure 1.3). These effects disappear within 500 m. There is wide variation in both ammonia and total nitrogen deposition depending on proximity to point sources (Pearson and Stewart, 1993). In addition to this, ammonia emissions also vary seasonally, peaking in spring and lowest in winter (ApSimon, 1987) so estimates are not always reliable.

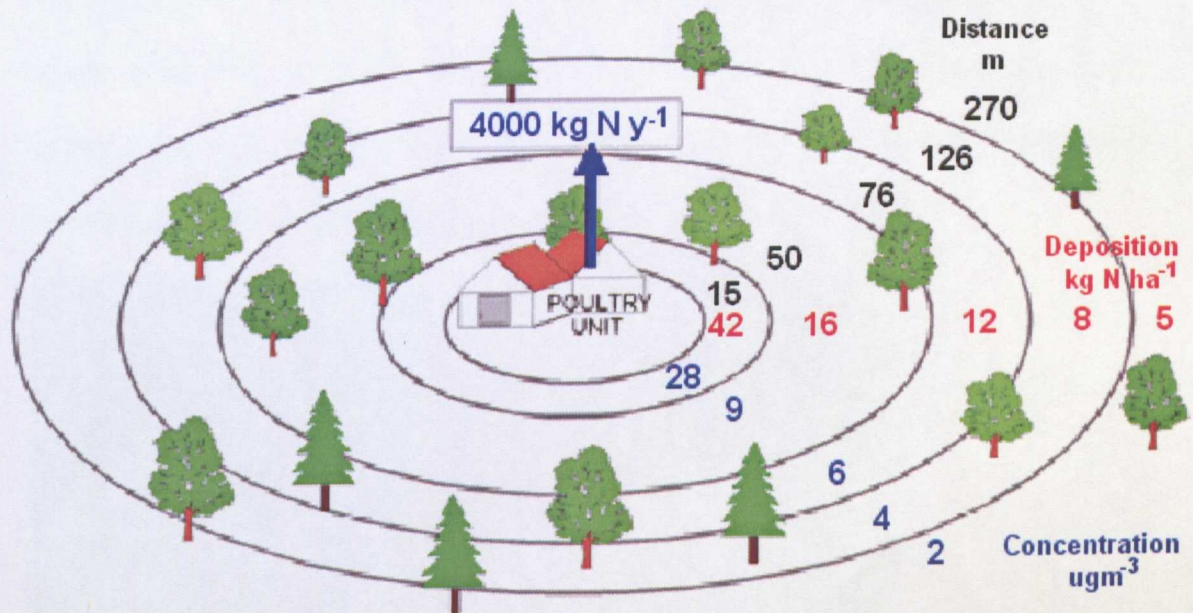


Figure 1.3 Farm scale ammonia emission and deposition (Fowler *et al.*, 1998).

Emissions of oxides of nitrogen in the UK primarily arise from the combustion of fossil fuels, but also from biomass burning, lightning, NH₃ oxidation, microbial soil processes such as denitrification, stratospheric input and marine photolytic and biological processes (Pitcairn *et al.*, 1995). NO concentrations are highest in the urban

environment, in cities and near roads (Stulen *et al.*, 1998). NO_x ($\text{NO} + \text{NO}_2 + \text{NO}_3$) is also a short-lived gas, with a lifetime of 1-10 days (Oliver *et al.*, 1998).

At a European level the problem is both serious and widespread. In 70% of countries in Europe more than half of the terrestrial ecosystem areas are affected by eutrophication, a situation which has improved little since 1990 (EMEP, 2000). Despite this, northern European countries such as Norway and Latvia have benefited from reductions in transboundary nitrogen pollution (EMEP, 2000). The highest concentrations of nitrate in air are found in central and southern England, northern France, Belgium, the Netherlands and western Germany. Ammonia is found at highest concentrations in Belgium, the Netherlands and the Ukraine as is shown in figures 1.4 and 1.5. The relative importance of the two components differs from country to country, region to region.

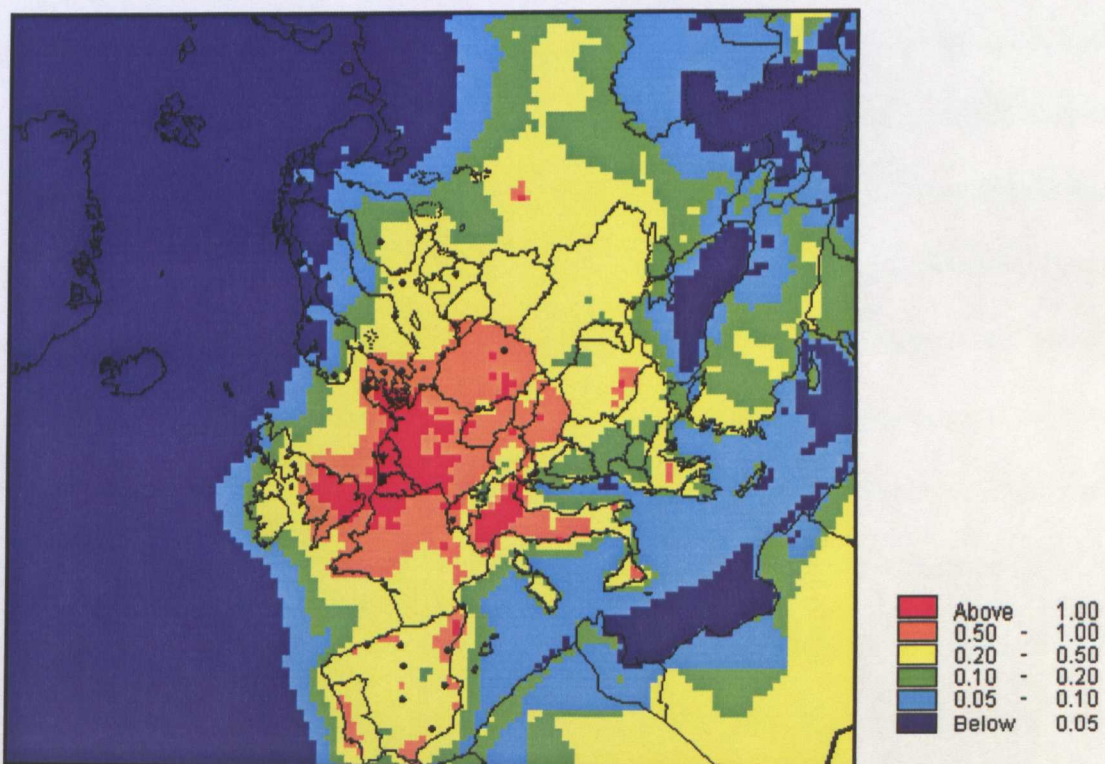


Figure 1.4 Yearly averaged total nitrate concentrations in air ($\mu\text{g N m}^{-3}$) (EMEP, 2001)

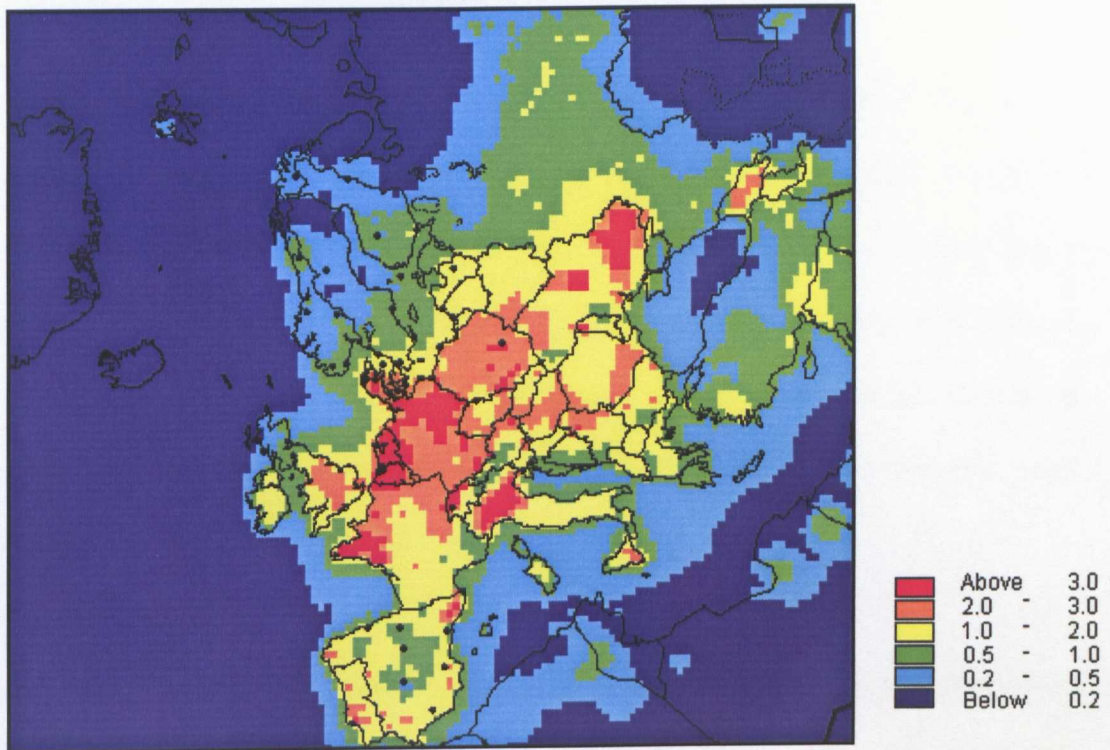


Figure 1.5 Yearly averaged total ammonia concentrations in air ($\mu\text{g N m}^{-3}$) (EMEP, 2001).

At Rothamsted Experimental Station, Harpenden, current levels of nitrate deposition in the winter months approach those common in the late 19th century, while current spring values are much higher (Brimblecombe and Pitman, 1980). It is estimated that by the year 2050 there will be an increase in nitrogen and phosphorus- driven eutrophication by 2.4-2.7 times in terrestrial, freshwater and near-shore marine ecosystems world-wide (Tilman *et al.*, 2001). From 1992 the introduction of catalytic converters reduced emissions of NO_x from new cars, and total emissions from road transport gradually declined as the number of cars with catalysts increased (RGAR, 1997). Despite this, concentrations of oxidised and reduced nitrogen in UK precipitation and hence wet deposition of N have increased during the last 30 years (Pitcairn *et al.*, 1995).

1.1.2 The effects of nitrogen deposition

The effects of nitrogen deposition on vegetation and soils are varied and can be considered a major threat to the functioning of natural and semi-natural ecosystems (Bobbink *et al.*, 1998). The different effects on vegetation can be divided into a number of classes: direct toxicity, increased susceptibility to pests and disease, increased susceptibility to environmental stressors and soil-mediated effects of acidification and eutrophication effects. Amongst these, acidification and eutrophication are globally the most important.

Many plant species in semi-natural ecosystems are adapted to low nutrient conditions and can compete successfully only in nitrogen-deficient soils (Grime, 1979). Increased fixed nitrogen deposition over recent decades provides excess available nitrogen that may cause changes in ecosystem species composition.

Both atmospheric deposition and natural nutrient cycling processes contribute to ecosystem acidification. However, while internal ecosystem cycling of nutrients generates and consumes large quantities of H^+ , many of these processes balance each other (Sutton *et al.*, 1993). By adding excess N we are disrupting this process.

The results of research to date shows there is clearly great potential for the deposition of nitrogen to have an adverse effect on semi-natural habitats causing eutrophication, soil acidification and consequent changes in soil chemistry, to affect plants directly and to cause a decline in species richness. The evidence for each habitat type will now be reviewed.

1.1.2.1 Forests

Publications concerning forest damage in Western Europe have expressed considerable alarm, particularly in Germany where the vitality of the forests has decreased rapidly. Air pollution is generally believed to be the main cause (Tomlinson, 2003; Matzner and Murach, 1995; Roelofs *et al.*, 1985). Acid pollutant inputs have decreased and this is no longer of such great concern however it is conceivable that these losses could occur again in the future if inputs increase again (Tomlinson, 2003). Three types of study have been conducted to investigate the effects of nitrogen deposition on forest vegetation: (1) comparisons of old and new vegetation samples from permanent or semi-permanent plots, (2) nitrogen fertilisation experiments, and (3) regional comparisons along deposition gradients. Despite a number of years of intensive research, many conflicting opinions about the long term effects of acidic deposition on European forests have been expressed (Binkley and Högberg, 1997) and the response of forest ecosystems in Europe to chronic N deposition has been found to be highly variable (Dise *et al.*, 1998). Despite this some Europe-wide trends have been identified. A survey of European forests by Dise and Wright (1995) showed that nitrate leaching was negligible below a threshold of 10 kg N ha⁻¹ yr⁻¹, leaching occurred at some sites with deposition ranging from 10-25 kg N ha⁻¹ yr⁻¹ and was found in all sites with deposition greater than 25 kg N ha⁻¹ yr⁻¹. This was expanded by MacDonald *et al.* (2002) who examined 181 forests throughout Europe. They found a strong relationship between N deposited in throughfall and nitrate leaching. High nitrate leaching occurred at all sites with a pH of less than 4.5 and N inputs of over 30 kg N ha⁻¹ yr⁻¹. Studies carried out to investigate acidity of forest soils have documented a decline in the pH of soils over the last few decades

(Binkley and Högberg, 1997) and an increase in forest growth has been observed (Nellemann and Thomsen, 2001) which may also be related to enhanced N inputs.

There has been concern about other effects of N deposition associated with N cycling and eutrophication. Brunet *et al.* (1998) used a deposition gradient from southern-most Sweden where deposition is high towards the north-east to investigate N deposition effects on forest understorey vegetation. The south-west coast of Sweden receives as much as 25 kg-N ha⁻¹ year⁻¹; other parts of southern and central Sweden receive between 5 and 10 kg-N ha⁻¹ year⁻¹ (Binkley and Högberg, 1997).

Regression analyses combined with Canonical Correspondence Analysis (CCA) were used to show much higher rates of nitrogen mineralization and nitrification in Skåne, southern Sweden, especially for the moist acid soils, than in the other study areas. The CCA ordinations show a floristic gradient from forest stands with a higher share of acid-intolerant woodland species and species with low nitrogen demands in the eastern and north eastern study regions to a concentration of acid-tolerant and nitrophilous species in Skåne. Unfortunately the close correlation between longitude/latitude and nitrogen deposition in the study region did not allow a full separation of the effects of nitrogen deposition and the effects of other climatically controlled factors such as growing period (Brunet *et al.*, 1998). Effects of increased N deposition may also critically depend on soil type (Sonnleitner *et al.*, 2001).

By examining eleven areas along a regional N deposition gradient in New England, McNulty *et al.* (1991) identified several relationships between N deposition and foliar and forest floor chemistry. A negative correlation with the lignin:N ratio in foliage

was significant across the gradient. This is transferred to the forest floor through litter fall and decomposition. There is a net increase in total forest floor N with increasing deposition. Forest floor N concentrations were significantly correlated with nitrification potential. An interaction between N deposition and forest floor Ca, Cu and Mg, and between N deposition and foliar concentrations of Ca and Mg was also found. No correlations were found between N deposition and rates of N mineralization or nitrification (McNulty *et al.*, 1991).

Using data from a number of database sets including NITREX, Dise *et al.* (1998) also found a significant relationship ($r^2=0.40$, $N=37$, $p=0.001$) between pine-needle N concentration and throughfall NH_4^+ -N flux (Figure 1.6).

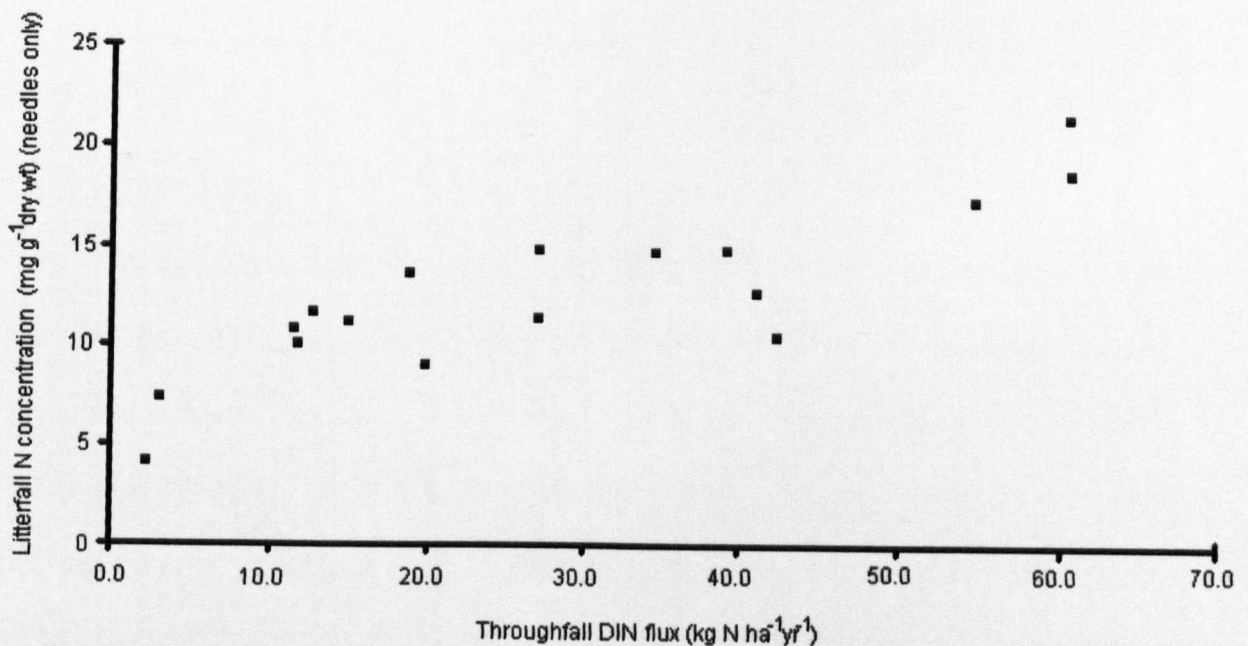


Figure 1.6 Relationship between N concentrations of pine needles (mg N g^{-1} dry wt) and throughfall flux of inorganic N ($\text{kg N ha}^{-1} \text{yr}^{-1}$) (Dise *et al.*, 1998).

Research looking at the N content of *Pinus nigra* var *maritima* needles using nutrient release/uptake experiments has shown that they take up ammonium from $(\text{NH}_4)_2\text{SO}_4$

solutions and compensate by excreting potassium, magnesium and calcium (Roelofs *et al.*, 1985).

The strong influence of point sources of N pollution on local ecosystems has been demonstrated in forests. Pitcairn *et al.* (1998) investigated the effects of localised sources of reduced N in the UK from intensive animal husbandry on woodland ground flora. Ammonia concentrations at the farms investigated were found to be large in the immediate vicinity of farm buildings. This was reflected in a high N content in mosses and herbs close to the livestock buildings. They found diversity was generally reduced closest to livestock buildings and there was an increase in nitrophilous species such as *Deschampsia flexuosa* and *Holcus lanatus*. Nitrogen sensitive species such as *Oxalis acetosella* and *Galium odoratum* became increasingly abundant with distance from the point source.

1.1.2.2 Heathland

20 years ago the serious effects of nitrogen deposition on heathland ecosystems had already been recognised in the Netherlands. In 1983 80 km² of heathland had become dominated by nitrophilous grass species, especially *Molinia caerulea* (Heil and Diemont, 1983). The accumulation of nitrogen in the ecosystem was found to be one of the main driving variables determining the rate of succession. In the Netherlands the input of nitrogen by atmospheric deposition to many ecosystems is extremely high as a result of intensive agriculture (Berendse, 1990). In a field experiment applications of fertiliser alone at a rate comparable to the deposition were found not to lead to the previously observed increased growth of grasses, but when combined with the impact of the heather beetle (*Lochmaea suturalis*), which was a key factor, effects

were evident (Brunsting and Heil, 1985). This may be an experimental artefact as a result of insufficient time over which N was applied. The interaction of nitrogen deposition and heather beetle attacks as trigger factors for the transition from heathland to grassland have also been reported by Heil and Bobbink (1993). Increased nutrient availability in the soil results in an upsurge in the nutrient content of the leaves. This increases the quality of food for the heather beetle stimulating the frequency and severity of attacks opening the canopy and allowing grasses to invade (Brunsting and Heil, 1985). A high nutrient content of *Calluna vulgaris* leaves has also been identified in the UK where Pitcairn *et al.* (1995) found that the spatial distribution of tissue N content of selected bryophytes and *Calluna* is related to the pollutant climate (figure 1.7).

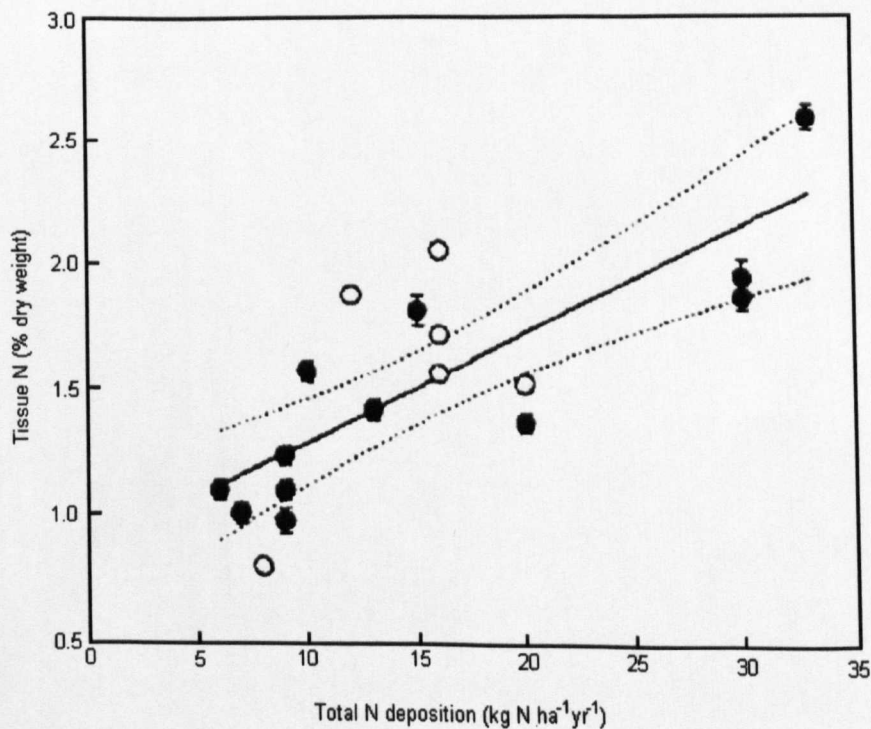


Figure 1.7 Relationship between average tissue N content of *Calluna* and total N deposition at range of sites in the UK. Unfilled circles represent unreplicated estimates (Pitcairn *et al.*, 1995).

In north Wales a long-term nitrogen addition experiment also demonstrated negative effects of nitrogen enrichment. The first stage of the application experiment showed an apparently positive effect on *Calluna* with increased shoot growth and flowering. During the same period the mycorrhizal community was unaffected. Five years into the nitrogen applications there were, however, clear signs that continued nitrogen applications could lead to adverse effects including a loss of the positive growth response. These experimental applications of small amounts of nitrogen (0.1, 0.8 and 12 kg N ha⁻¹ yr⁻¹) have demonstrated increased incidence of late-winter injury and a substantial reduction and complete removal of the understorey layer of bryophytes and lichens (Carroll *et al.*, 1999) (figure 1.8).

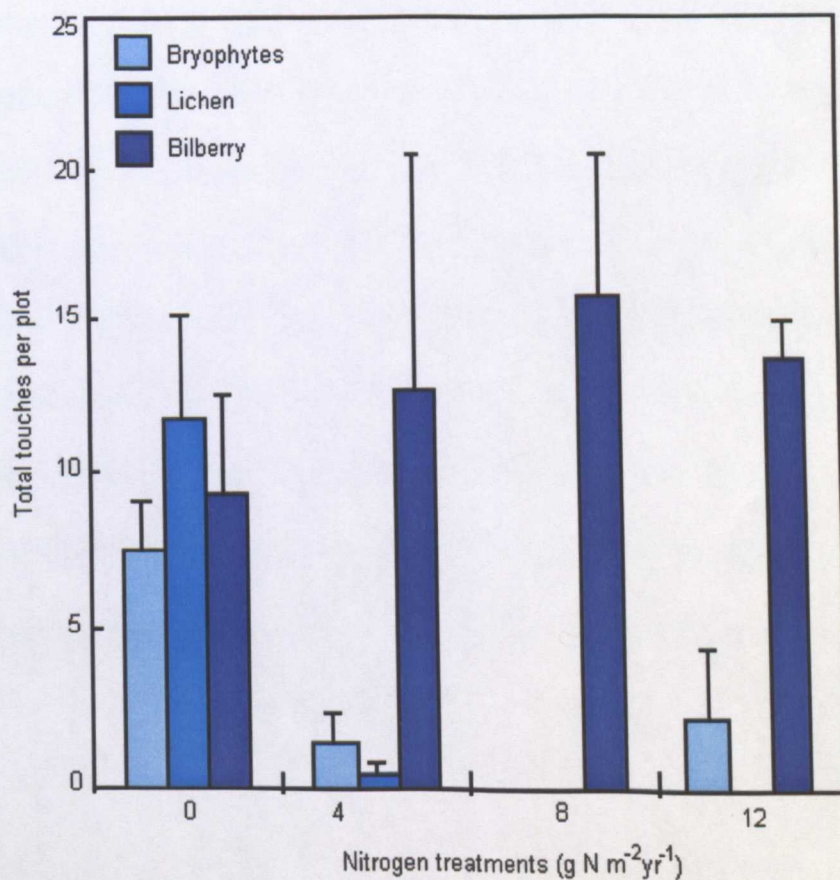


Figure 1.8 Effect of long-term additions of NH₄NO₃ on the abundance of understorey species on *Calluna vulgaris* moorland plots. Total touches per plot for bryophytes, lichen and bilberry (Carroll *et al.*, 1999).

Power *et al.* (1998) found experimental plots treated with nitrogen additions of 15.4 kg N ha⁻¹ yr⁻¹ had nearly 30% more litter accumulated on the soil surface than control plots and a faster rate of litter turnover. Nitrogen additions equivalent to a doubling of background deposition levels have a substantial impact on the productivity of *Calluna* in this nutrient-poor system.

A further nitrogen addition experiment carried out by Aerts *et al.* (1990) demonstrated the role of competition in the decline of heathland vegetation. They found that at low nutrient levels (N,P,K = 5, 1, 5 g m⁻² yr⁻¹) *Molina* was out-competed by both *Erica* and *Calluna*, however, at higher levels of nutrients (N,P,K = 20, 4, 20 g m⁻² yr⁻¹) the higher potential growth rate of *Molina* allows it to out-compete other species, which would cause a reduction in diversity. Airborne ammonium deposition on heathland soils leads to a succession from heather-dominated to grass-dominated heathlands. The nitrogen levels in grass-dominated heathland soils have been found to be much higher than *Calluna*-dominated ones, however the study does not prove N was the direct cause of the increase in domination of grasses (Roelofs, 1986). Roem *et al.* (2002) identify increased aluminium ion concentrations in the upper soil layer caused by acidification of soils to be the most important factor causing a decline in species richness.

1.1.2.3 Mires

In many semi-natural ecosystems the atmospheric input may represent only a small proportion of the total available nitrogen, but since the bryophyte component of the vegetation is often dependent on an atmospheric source of nutrients they are

especially sensitive to nitrogen deposition. Bryophytes are the dominant vegetation type in ombrotrophic mires (Press *et al.*, 1986, Hogg *et al.*, 1995) with plants that are primarily adapted to utilise the low levels of nitrogen present naturally.

Hogg *et al.* (1995) working at Askham bog, Yorkshire over a period of 13 years found that of the 27 most abundant species of ground flora at the site, 10 species declined in cover and only three increased. Several species in the most base-rich areas were found to decline while *Urtica dioica*, a strongly nitrophilous species, became more abundant. A nitrogen addition experiment on an ombrotrophic blanket mire in Migneint, north Wales, showed that nitrogen additions of $12 \text{ kg N ha}^{-1} \text{ year}^{-1}$ severely inhibited the growth of several *Sphagnum* species. There is a close association between the regional variation in moss tissue nitrogen concentrations and atmospheric nitrogen deposition patterns (figure 1.9) (Baddeley *et al.*, 1994).

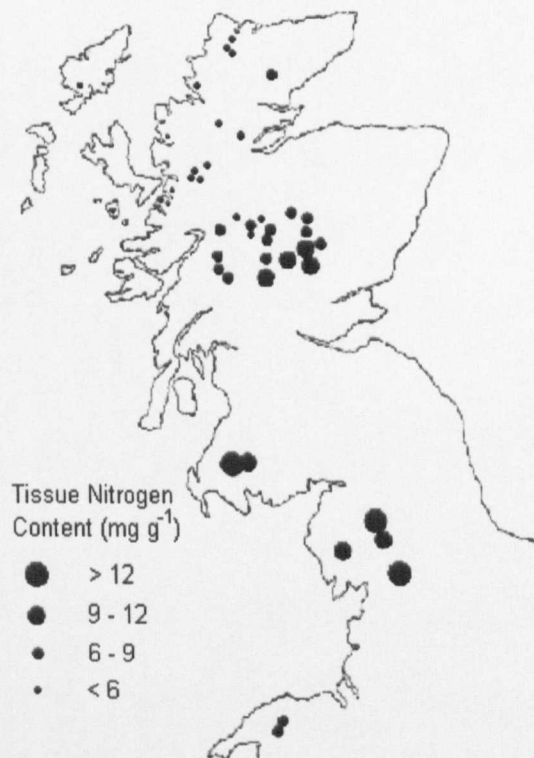


Figure 1.9 Nitrogen content (mg g^{-1} dry weight) in *Racomitrium lanuginosum* from mountain summit sites in north-western Britain (Baddeley *et al.*, 1994).

A significant increase of 62% in tissue nitrogen has been found during the last 30 years in the ombrotrophic *Sphagna* species examined at Moor House National Nature Reserve (Pitcairn *et al.*, 1995). These mosses depend largely on rainfall for their nutrient supply. The minerotrophic groups of *Sphagna*, which receive most of their nutrients from groundwater, showed no increase in their tissue N (Pitcairn *et al.*, 1995). This change has been accompanied by the loss of certain bryophyte and lichen species determined by repeated survey of permanent plots (Pitcairn *et al.*, 1991). Tomassen *et al.* (2003) found a linear response of tissues N concentrations in *Sphagnum fallax* to experimental nitrogen addition.

Peat soils are very sensitive to acidification. In organic soils acid precipitation leaches cations, which are replaced on cation exchange sites by H^+ ions. In soils with a thin organic layer these cations can be replaced by element cycling from underlying mineral layers, but in thick peat soils this process is not so effective (Heal and Smith, 1978). Skiba *et al.* (1989) found a strong correlation between acid deposition and peat acidity in Scotland. They found that peats with the highest acidity (pH less than or equal to 3) and lowest base saturation ($\leq 10\%$) were mainly found where acid deposition was greater than $0.8 \text{ kg } H^+ \text{ ha}^{-1} \text{ yr}^{-1}$.

Working in calcareous fens, Bergamini and Pauli (2001) also demonstrated a reduced mass of bryophytes and a decline in bryophyte species density with the addition of N alone ($100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ as NH_4NO_3) and in an NPK fertilisation (N:P:K = 2:1:3; $\text{NH}_4\text{NO}_3 + \text{P}_2\text{O}_5 + \text{K}_2\text{O}$; $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Typical species of fens in the Netherlands have declined in response to nitrogen deposition, and fast growing

species including *Sphagnum squarrosum* and *Polytrichum commune* have increased (Paulissen *et al.*, 2003).

1.1.2.4. Grasslands

There has been a pronounced loss of unimproved grasslands and a consequent decline in many of their less common and specialist species in western Europe. This has occurred mainly during the second half of the last century with conversion to high input-low diversity farming systems (Blackstock *et al.*, 1999). In addition to this, approximately 60% of lowland grassland, heath and scrub key conservation sites (as identified by Ratcliffe, 1977) receive more than 20 kg N ha⁻¹ year⁻¹ as atmospheric deposition. Unfortunately there is no effective way of protecting conservation sites against existing levels of N deposition (Woodin and Farmer, 1993).

The impact of N deposition on semi-natural grasslands, in terms of acidification and eutrophication, is influenced by the amount of nitrogen deposition, duration of the deposition, the buffering capacity and nutrient status of the soil and the management regime. Different types of grassland therefore differ in their sensitivity to atmospheric N, with those on poorly buffered acidic soils being more vulnerable than those on more highly buffered calcareous soils (Chalmers, 2000). Despite this there has been considerably more research investigating nitrogen impacts on neutral grassland (semi-natural swards lacking any pronounced calcicole or calcifuge elements (Tansley, 1911, 1939)) and calcareous grasslands (calcicolous (lime-tolerant) species are a dominant feature (Rodwell, 1992)) than acidic grasslands (calcifugous (lime-intolerant) species are a dominant feature (Rodwell, 1992)).

Primary amongst the investigations into the effects of nitrogen additions to grasslands is the Park Grass nitrogen addition experiment at Rothamsted Experimental Station, Hertfordshire, UK (Jenkinson *et al.*, 1994). Lawes and Gilbert initiated a range of nitrogen additions in 1856 on an area that had already been under grass for several centuries. Large differences have developed in the vegetation yields of the various plots as the result of fertiliser and lime treatments (Jenkinson *et al.*, 1994). Six years after the beginning of the experiment some changes had already been observed. Applying fertiliser as sodium nitrate at a rate of $48 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ or more led to a disappearance of legumes, and species numbers decreased from around 50 to approximately 35 within a few years. The results of the nitrogen addition experiment show clear and well documented effects of eutrophication and soil acidification on species number and composition (Goulding *et al.*, 1998) (figure 1.10).

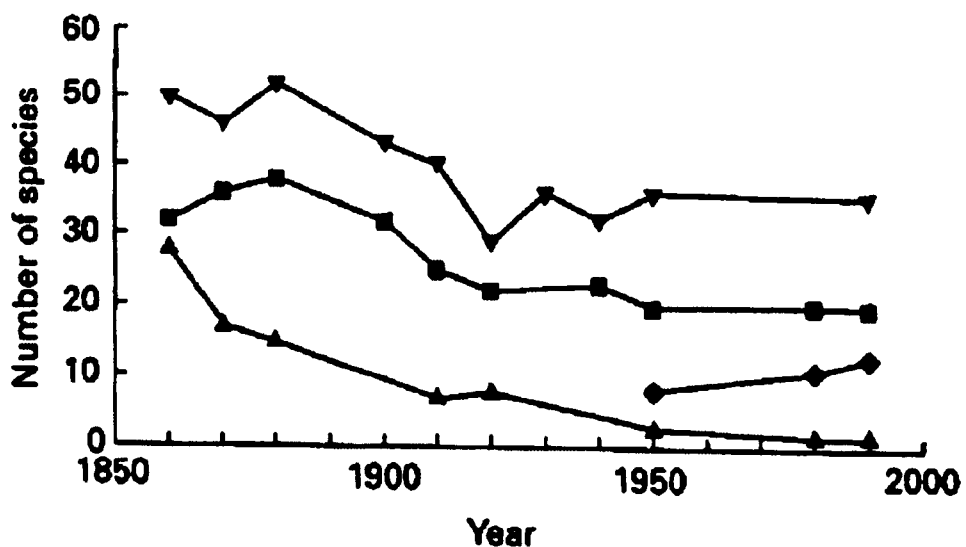


Figure 1.10 Changes through time in the number of species on the Park Grass Experiment. ▼ no fertiliser; ■ sodium nitrate; ▲ ammonium sulphate, no lime; ◆ ammonium sulphate with lime (Goulding *et al.*, 1998).

The unmanured plots at Park Grass, with 50 – 60 species, remain the most diverse species assemblages, including many broad-leaved plants (AFRC, 1991). Analysis of long term trends in yield carried out by Jenkinson *et al.* (1994) found no evidence of a response to increasing atmospheric inputs of nitrogen. This suggests that those looking for such changes may have to wait a long time before they become detectable in the field. In the grassland plots that have not had fertiliser addition, the pH of the surface soil and subsoil decreased due to atmospheric acid deposition (NO_x , NH_4 and SO_2). Taking the ratio of external:internal proton inputs Blake *et al.* (1999) demonstrated that no more than 50–60% of the acid inputs at Park Grass were as acid deposition, the rest coming from sources including N transformations, base cation uptake and organic acid. Atmospheric deposition may cause as much as 30% or more of soil acidification, the largest source comes from the dissociation of carbonic acid and other acids formed by the mineralization of C, N and S (Johnston *et al.*, 1986).

Berlin *et al.* (2000) working in Sweden also found an increase in graminoid species between 1966 and 1990 in 8 semi-permanent plots. The initial reduction in species diversity was as a result of fertiliser application, but the consequent and steady decrease had a common cause across all the plots – most likely atmospheric deposition.

Application of nitrogen at even low levels can lead to adverse effects. $25 \text{ kg ha}^{-1} \text{ N year}^{-1}$ as ammonium nitrate encouraged the spread of agriculturally productive grasses in hay meadows at Tadham Moor, Somerset within 2 years, and $50 \text{ kg ha}^{-1} \text{ year}^{-1}$ significantly reduced species richness within 3 years (Mountford *et al.*, 1993). Fertiliser addition changed the sward to one dominated by grasses at the expense of

forbs, *Carex* and *Juncus* species (Kirkham and Wilkins, 1994). Very few forbs maintained or increased their cover with the addition of N (Kirkham and Wilkins, 1994a). After 5 years of fertiliser application, the balance of species in the seed bank had been changed in favour of species that were more competitive under fertile conditions (Kirkham and Kent, 1997). The ecology of these meadows has been described as “sensitive to even small levels of fertiliser application” (Kirkham and Wilkins, 1994). Despite the effects of nitrogen addition, in a separate experiment where various levels of N and P were also applied Kirkham *et al.* (1996) found phosphorus (P) to be the most influential element, with several species changes associated with its addition. This suggests that the habitat is P limited rather than N limited, which could be an effect of nitrogen deposition.

Morecroft *et al.* (1994) applied ammonium nitrate to both calcareous and acidic grasslands in the Peak District. They found that calcareous grassland plant growth was co-limited by phosphorus and nitrogen supply. Changes in vegetation composition due to nitrogen addition were not observed. This could be a result of the calcareous soils being very well buffered against changes in pH and the co-limiting nutrient, phosphorus, not being supplied. Wilson *et al.* (1995), working on experimental trials using calcareous soils, reported increased productivity and changes in species composition with additions of ammonium sulphate and nitrate-N as nitric acid and sodium nitrate at a rate of $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for 2 years. In this case the results suggest that the grasslands were nitrogen limited, possibly due to lower background N deposition than in the Peak District.

In acid grasslands Morecroft *et al.* (1994) initially found the ammonium nitrate addition caused a decline in *Rhytidiadelphus squarrosus*, but no other change in the vegetation composition. After 6-7 years there was a small decrease in higher plant cover and reduced bryophyte cover. Soil nitrogen mineralization was stimulated by nitrogen addition (Lee and Caporn, 1998). Most of the nitrogen was immobilized in the soil rather than being lost by leaching or volatilisation (Phoenix *et al.*, 2003). It was also found that leaf and shoot nitrogen concentration and plant nitrate reductase activity increased with increasing nitrogen addition (Morecroft *et al.*, 1994).

In a correlative study spanning England and Wales, Kirkham (2001) also found an increase in plant nitrogen content for some acid grassland species with increasing N deposition. Samples of shoot tissues were taken from upland sites in England and Wales in 1996 and 1997 and analysed for nitrogen content, annual net assimilated N per hectare (calculated using tissue N concentration combined with dry matter yield) and N:P ratio. Six groups of species were sampled: *Calluna vulgaris*, *Vaccinium myrtillus*, *Molinia caerulea*, *Nardus stricta*, *Eriophorum vaginatum* and an *Agrostis/Festuca* mix. N content as a percentage of shoot dry weight of mature *Calluna* was found to be correlated with NO_x-N deposited during 1996. The regression equation showed an increase in shoot N concentration of 0.27 mg g⁻¹ for each additional kg NO_x-N ha⁻¹. Shoot percentage N for all *Calluna* growth phases pooled showed no significant overall relationship with N deposition in either of the years surveyed. Shoot N concentration in *Agrostis/Festuca* was significantly correlated with NO_x deposition in 1997 (figure 1.11).

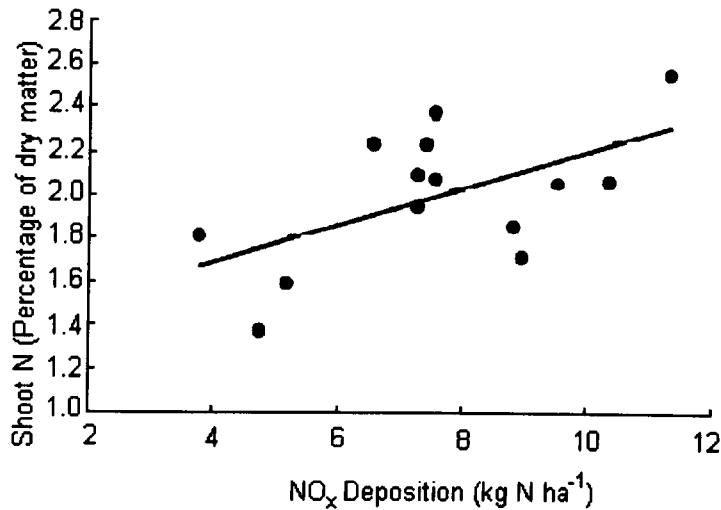


Figure 1.11 Relationships between N deposition and shoot N content in live shoot tissue of *Agrostis/Festuca* vegetation ($r^2=0.28$; $p<0.001$) (Kirkham, 2001).

In the same study N was limiting at most *Agrostis/Festuca* sites. However other species showed changes in tissue N concentration indicating that the increases in atmospheric N deposition that have occurred over recent decades have changed nutrient dynamics of many upland regions into systems limited by P (Kirkham, 2001).

Wedin and Tilman (1996) also found losses of diversity at nitrogen addition levels of between 10 and 50 kg N ha⁻¹ yr⁻¹ in Minnesota grasslands together with large shifts in the species composition. This was accompanied by changes in the C:N ratio of plant tissues, increased nitrogen mineralization and increased soil nitrate.

1.1.3 Legislation

In 1994 the Oslo Protocol on Further Reduction of Sulphur Emissions formally introduced the concept of 'critical loads' for air pollution legislation. A critical load is defined as 'the highest deposition of acidifying compounds that will not cause

chemical changes leading to long term harmful effects on ecosystem structure and function (Nilsson and Grennfelt, 1998). The current critical load for nitrogen deposition is $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for acid grasslands (Hall *et al.*, 1998), however there is much uncertainty regarding the accuracy of critical loads for nitrogen deposition (NEG-TAP, 2001) especially since nitrogen can both acidify and increase eutrophication of terrestrial ecosystems. Several methods can be used to calculate critical loads for nitrogen (Kuylensstierna *et al.*, 1998) giving different results. However, it is clear that substantial areas across Europe may be at risk from the effects of nitrogen deposition.

The importance of nitrogen as a pollutant in Europe was recognised with the 1988 EU Protocol concerning the control of emissions of nitrogen oxides, which aimed to halt emissions at their current levels. This was followed by the 1999 Gothenburg Protocol to Abate Acidification, Eutrophication and Ground-level Ozone. The protocol sets maximum permitted levels for the emission of four pollutants including NO_x and ammonia. NO_x emissions should be cut by 41% compared to 1990 levels and ammonia emissions by 17% by 2010. It is hoped that once the protocol is fully implemented, areas with excessive levels of acidification and eutrophication will be reduced considerably.

The EU Water Framework Directive (2000/60/EC) has further implications for nitrogen pollution. The directive, which was passed by the council of Europe in December 2000, aims to prevent deterioration of, and ultimately enhance, ecological water quality and prevent ground water pollution. The directive would thus require that fertiliser run-off and nitrogen leaching be controlled or prevented.

Biodiversity legislation is also very important within the remit of this study. The Habitats Directive (92/43/EEC) is one of the strongest pieces of legislation regarding the protection of biodiversity. The directive aims to maintain biodiversity through the conservation of natural habitats and wildlife. This purpose is pursued via the designation of sites based on species and habitats listed in the directive, where habitat integrity must be protected by member states.

1.2. The need for this study

The recent legislation concerning pollutant nitrogen has highlighted its importance. However, despite the work that has already been done in this field, there are still many questions that remain to be answered. Experimental addition of nitrogen to semi-natural ecosystems has shown the *potential* for nitrogen deposition to change vegetation composition and soil chemistry, but little is known about the *actual* effects of nitrogen deposition as it is currently occurring in the UK. With few long term and widespread datasets, detecting change at an ecosystem level is exceptionally difficult. Detecting change in ecological systems before it is too late is also very hard (Pacala *et al.*, 2003). There is even less known about the processes behind the changes that are occurring and the ability of natural systems to recover.

The concept of critical loads, central to current legislation, remains controversial and current critical loads are uncertain and consequently open to much criticism. Although they take a habitat-specific approach, some habitats are insufficiently studied for accurate estimates. There is a clear need for further work in this area and the present project aims to address some of the issues raised.

1.3. Aims and Objectives

The main aim of this project is to determine whether a relationship exists between levels of ambient nitrogen deposition in the UK and a) vegetation community structure, b) soil and vegetation mineral content and c) soil processes. The results will then be used to identify indicators of pollution damage in soil and vegetation and relate these to the deposition of nitrogen. The aims of the project can be broken down as follows:

- a) To identify changes in community composition as a result of eutrophication and acidification using established indicator values (Hill *et al.*, 1999) and to identify species that are especially sensitive to nitrogen inputs.
- b) To identify changes in the soil chemical properties as a result of eutrophication and acidification.
- c) To identify changes in the vegetation chemical properties as a result of eutrophication and acidification.
- d) To identify changes in the mineralization rate in soils as a result of eutrophication.
- e) To identify changes in nutrient limitation in acid grassland ecosystems as a result of eutrophication.

1.4. Thesis layout

This thesis will be arranged into a further 6 chapters. Chapter 2 will describe the selection of a plant community for this investigation and the field methods used. Chapters three to five will investigate the effects of nitrogen deposition on plant communities (chapter 3), soils chemical properties and processes (chapter 4) and plant tissues (chapter 5). Chapter 6 draws the preceding studies together looking at the ecosystem as a whole and the effect of nitrogen deposition on the interactions between plants and the soil. Chapter 7 summarises the conclusions of this study.

2. Field Methods

2.1 Choice of Vegetation Community

An initial decision was made to work on grasslands, as they have received less attention in terms of research into the impact of N deposition in comparison to forests or heathlands. In order to select the community type that would be most suited to this investigation, a close analysis of the National Vegetation Classification (NVC) (Rodwell, 1992) communities was made. The NVC provides a phytosociological account of British vegetation. The semi-natural vegetation in Great Britain is divided into a series of communities based upon a Two Way Indicator Species Analysis (TWINSpan) (Hill, 1979) of 35,000 samples taken from 80% of the 10 x 10 km squares of Britain. Forty-eight grassland and montane vegetation types, divided into three classes, are described in British Plant Communities Volume 3. The first class comprises of mesotrophic grasslands i.e. the 'neutral grasslands' as described by Tansley in 1911. The second class includes calcicolous grasslands i.e. those found on base-rich soils and the third contains calcifugous grasslands i.e. those found on base-poor, acid soils (for a definition of these terms see chapter 1.1.2.4) (Rodwell, 1992). By using a single community type for the investigation it is hoped that variation between the sites can be minimised.

There are many criteria that must be considered in the selection of the community. If a comparison is to be made between sites, it is important that they are as similar as possible in all respects except the one under investigation. The chosen community needs to be sensitive to the effects of nitrogen deposition and must also occur

throughout the range of nitrogen deposition found in Britain. These criteria and others will be discussed in order to conclude which is the most appropriate community.

2.1.1 Distribution

Nitrogen deposition to heathland and semi-natural grassland in Britain is shown in figure 2.1.

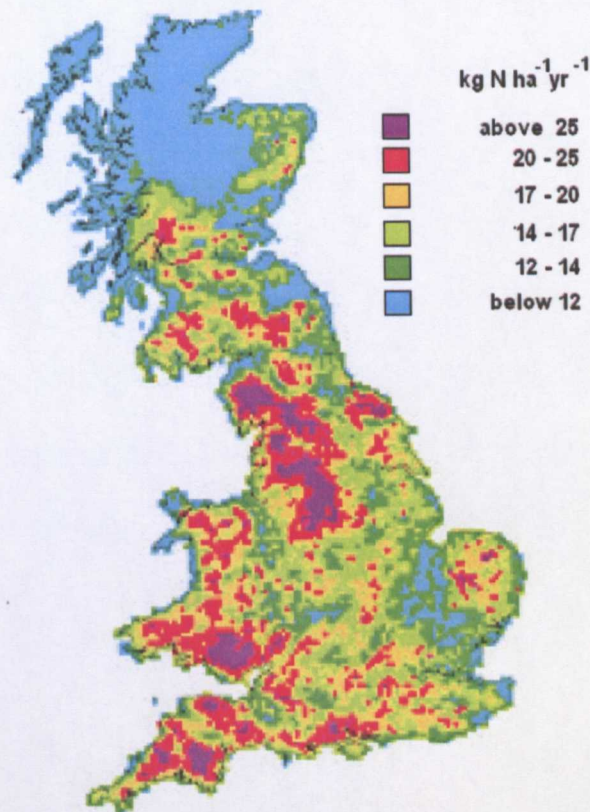


Figure 2.1 Total nitrogen deposition ($\text{kg N ha}^{-1} \text{yr}^{-1}$) to heathlands and rough grazing in the UK accounting for land cover (NEGTA, 2001)

To achieve coverage of this entire gradient, it is important that the community used is widespread. It is also desirable that the distribution of the grasslands should allow for examination of deposition levels in different areas of Britain to avoid confounding

factors associated with climate and latitudinal gradients, given the cooler, wetter climate in the north.

Many of the grassland communities have very restricted distributions, making them unsuitable for use in this investigation. This is especially true of the calcareous grasslands, for example CG9 *Sesleria albicans*-*Galium steneri* grassland is restricted to the Carboniferous limestone of the Morecambe Bay area and CG14 *Dryas octopetala*-*Silene acaulis* is only found in the Scottish highlands. The communities that are sufficiently widespread to be of use in this investigation are as follows: MG1 *Arrhenatherum elatius* grassland, MG5 *Cynosurus cristatus*-*Centaurea nigra* grassland, MG6 *Lolium perenne*-*Cynosurus cristatus* grassland, MG10 *Holcus lanatus*-*Juncus effusus* grassland, MG11 *Festuca rubra*-*Agrostis stolonifera*-*Potentilla anserina* grassland and U4 *Festuca ovina*-*Agrostis capillaris*-*Galium saxatile* grassland. Some of these grasslands are unsuitable for other reasons that will be discussed in the following sections. The distributions for MG1, MG5 and U4 are given in figure 2.2. A full description of the communities is provided in appendix 1.

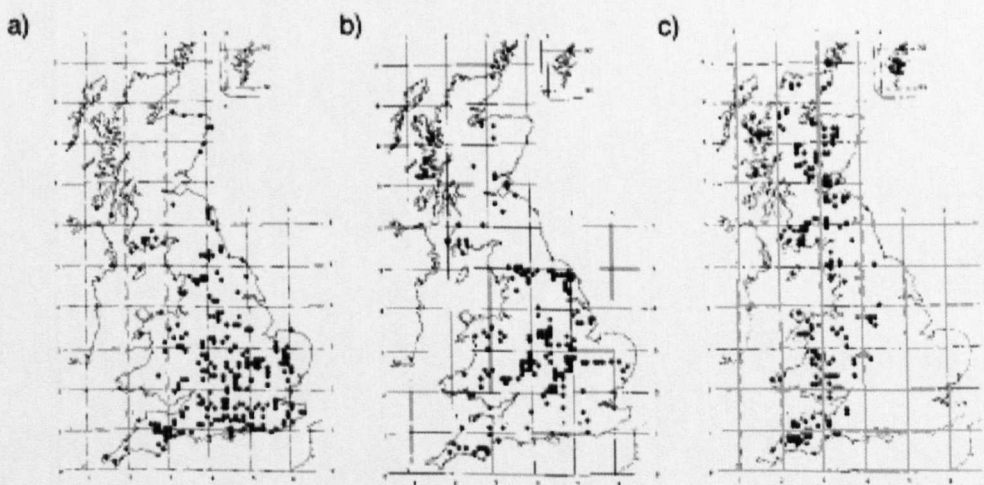


Figure 2.2 Distribution of a) MG1 grasslands, b) MG5 grasslands and c) U4 grasslands (Rodwell, 1992).

The distribution of MG5 grasslands is more extensive in England than suggested by figure 2.2 b. Blackstock (1999) identifies over 5000 ha of MG5 grassland in England and Wales (figure 2.3), principally made up of the MG5b (*Galium verum*) sub-community with a small proportion of MG5c (*Danthonia decumbens*) sub-community).

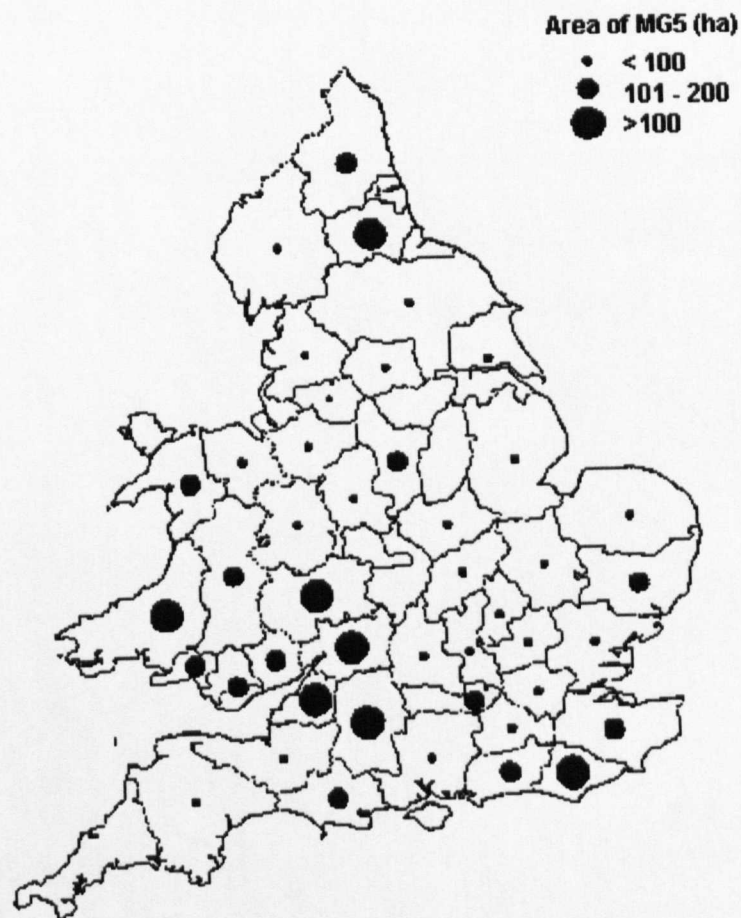


Figure 2.3 Cover of MG5 grassland in England and Wales recorded in surveys between 1978 and 1996 (Blackstock, 1999).

U4 grasslands cover between 3,000 and 5,000 ha in England and Wales alone, extending further into the south-east of England than the NVC survey suggests (Sanderson, 1998).

2.1.2 Hydrological Variability

Wet grasslands, or those that are seasonally wet, are unsuitable for this study because the water level has a profound influence on the soil mineralization rates in the soil (Stanford and Epstein, 1974) and consequently the availability of nitrogen to plants, and can lead to considerable within-site variability. This in turn affects the vegetation that is present. Water levels also affect the rate of nitrogen uptake by plants (McGill *et al.*, 1981). As MG10 grassland frequently occurs in mosaics with MG6 and M23 (a mire community) related to soil moisture (Rodwell, 1992), this makes it unsuitable, together with a number of other communities that were already eliminated. MG6 grassland is also found in some inundated soils, with a *Deschampsia*-dominated variant of the typical community being common (Rodwell, 1992)

2.1.3 Management

It is desirable that the grasslands should not be intensively managed and, since this investigation focuses on atmospheric N inputs, it is essential that they are not agriculturally improved. This consideration excludes the use of the grassland community MG11, which is frequently improved (Rodwell, 1992). It is also desirable that the management of the grasslands is not too variable between sites. The management of the 3 communities identified in 2.1.1 is summarised below:

MG1 grassland is ungrazed sward. The management of these grasslands is highly variable and they arise in many different situations ranging from road verges and building sites to abandoned pastures. These grasslands are subject to irregular mowing and an absence or irregularity of grazing. In some cases they can arise

through the agricultural improvement of MG5 grasslands as demonstrated in the Park Grass Plot experiments at Rothamsted (Brenchley and Warrington, 1958).

MG5 grasslands are typical hay meadows. They are traditionally managed with a hay cut taken between June and September and aftermath grazing in the autumn through to April. The exact timing for the hay cut and grazing varies between meadows. Most stands have a very long history of management, dating back many generations. The rich species diversity and attractive appearance of these meadows makes them valuable for nature conservation, although their distribution has been greatly reduced by agricultural intensification (Blackstock, 1999).

U4 grassland is a community maintained by grazing of stock, rabbits and deer. It is an important agricultural grassland making up a large portion of the rough grazing in Great Britain, especially in the upland fringes. Some of these pastures were established as early as the medieval period by clearing and burning, although management has changed considerably since. Stocking densities are variable depending on the quality of the grassland, with grazing predominantly by sheep using a 'put and take' regime – changing the grazing intensity to maintain the grassland in a suitable state (Hunter, 1962). The pattern of grazing by sheep has the effect of keeping vegetation short and varied (Rodwell, 1992).

2.1.4 Sensitivity to Eutrophication

Ellenberg scores (Ellenberg, 1979) provide a series of scores on a scale of 1-9 (sometimes 12) reflecting the environmental tolerances of plants. Examining the “nitrogen” (N) scores for the vegetation present allows an assessment of the

sensitivity of the grassland communities to the effects of nitrogen deposition. Hill *et al.* (1999) recently recalculated the original European scores devised by Heinz Ellenberg for British vegetation. The N value is, in effect, a general indicator of soil fertility. The N scale goes from 1 representing indicators of extremely infertile sites to 9 representing indicators of extremely rich fertile situations (Hill *et al.*, 1999). Average N for the component species of the community weighted for the frequency (Rodwell, 1992) of individual species are given in table 2.1 for each of the communities and sub-communities.

Community	Average Ellenberg score for N (Weighted according to frequency)	Range
MG1	5.01	2-9
MG1a	5.21	2-9
MG1b	5.69	2-9
MG1c	5.53	2-8
MG1d	4.64	2-9
MG1e	4.66	2-9
MG5	4.22	2-7
MG5a	4.33	2-7
MG5b	4.13	2-7
MG5c	4.06	2-7
U4	3.39	2-7
U4a	3.29	2-7
U4b	3.82	2-7
U4c	3.17	2-6
U4d	3.17	2-7
U4e	2.78	2-5

Table 2.1. Average Ellenberg N scores for MG1, MG5 and U4 sub-communities. Differences within and between communities in N indicator value are clearly shown in figures 2.4 to 2.7.

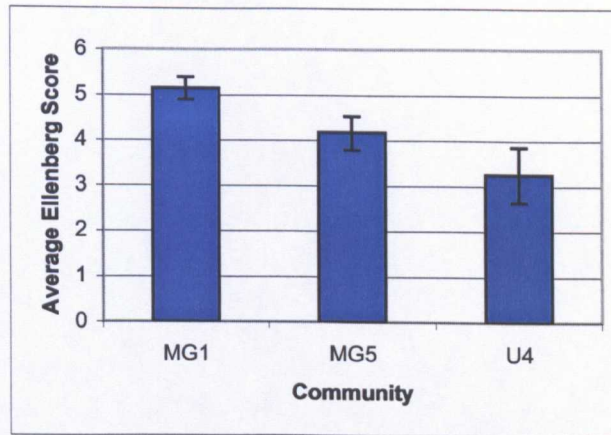


Figure 2.4 Mean Ellenberg N score for the different communities. Error bars show standard error of mean.

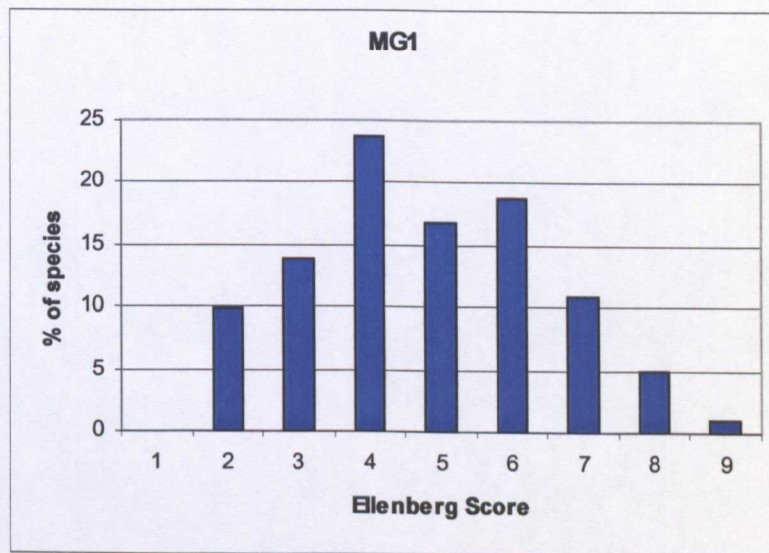


Figure 2.5 Distribution of Ellenberg N scores – MG1.

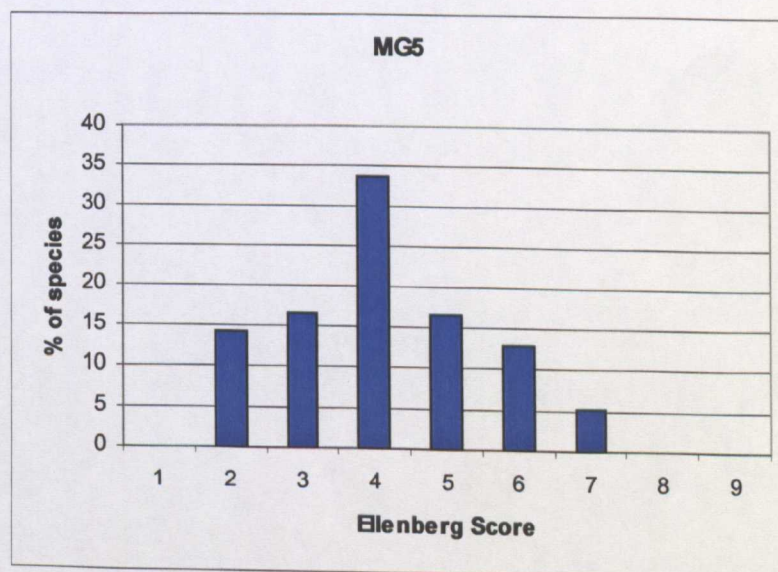


Figure 2.6 Distribution of Ellenberg N scores – MG5

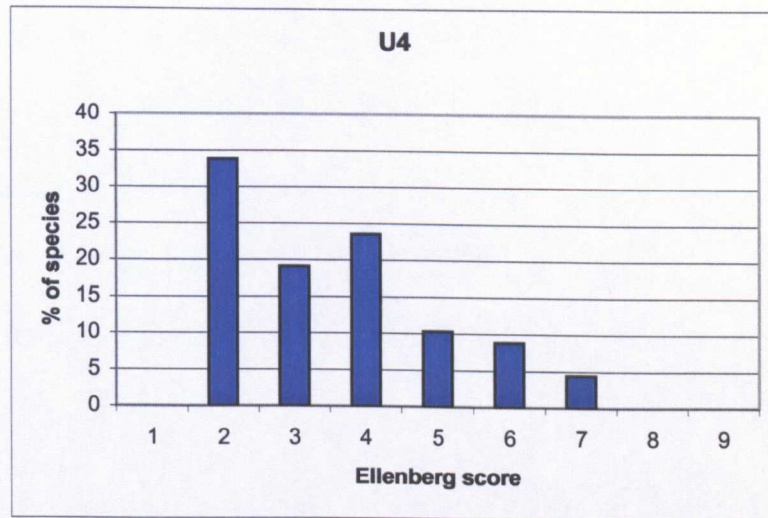


Figure 2.7 Distribution of Ellenberg N scores – U4.

U4 grassland is likely to be the most sensitive to nitrogen deposition as it has the greatest proportion of species that are indicators of infertile environments (77% with a score of 4 or lower). This is combined in the low weighted average (figure 2.4). This group also has the greatest variation in the Ellenberg N scores and the greatest variation in the scores between the sub-communities. This means that, although it is a community that is likely to be sensitive to nitrogen deposition, differences among sub-communities may mask changes due to differing rates of nitrogen deposition.

MG5 grasslands are also likely to be quite sensitive to nitrogen deposition as they have a minority of species of fertile environments (Ellenberg score of greater than 5) (35%) (figure 2.6). This is reflected in a relatively low mean Ellenberg score (figure 2.4). Mountford *et al.* (1993) found that the application of 25 kg N ha⁻¹ yr⁻¹ encouraged the spread of agriculturally productive grasses within 2 years in MG5. In general there was evidence of a change towards more species-poor mesotrophic communities under high rates of nitrogen application.

MG1 grassland shows a lower sensitivity to nitrogen with 53% of species indicating high fertility environments, indeed *Rumex obtusifolius* has an N score of 9. It is therefore less likely that these species would react to elevated nitrogen deposition. The presence of species with high Ellenberg scores for fertility and the high weighted average score combined with the management variability makes this community unsuitable for this investigation.

There is some variation between the sub-communities within a community. Figure 2.4 shows there is greatest variation between the sub-communities in U4 grassland. This is also shown in the variation in the ranges of the scores (table 2.1).

2.1.5 Soil Homogeneity

In order to detect any effects of nitrogen deposition on soil chemistry it is desirable for the soils to be as homogeneous as possible both between sites and within sites. U4 grassland is found on a range of acidic soils weathered from parent materials. These soils range from rankers through to brown earths, podzolic profiles to podzols proper, with mull or mor humus. The soils vary both within and between the sub-communities (Rodwell, 1992). Walker *et al.* (2001) showed that the soil pH of U4 grasslands can vary between sub-communities. U4a had a mean pH of 4.8 (4.3-5.0), U4b 5.0 (range 4.4-5.8) and U4c 5.0 (range 4.8-5.2). Data were not available for other sub-communities. Extractable phosphorus had a mean value of 0.4 mg kg⁻¹ for U4a, 1.6 mg kg⁻¹ for U4b and 0.3 mg kg⁻¹ for U4c. Potassium content was found to be variable between and within communities with a total range of 31-102 mg kg⁻¹.

MG5 grasslands have more consistent soils. These grasslands are found on brown soils with a loamy or clayey texture. MG5c, which extends up to the north of Scotland, can sometimes be found in slightly more sandy soils that have been improved by manuring. Walker *et al.* (2001) reported a mean soil pH for MG5c of 5.2 with a minimum of 5.0. For MG5a the mean pH is 5.4 and for MG5b 6.2. Phosphorus content covers less of a range than in the U4 community ($0.4 - 0.7 \text{ mg l}^{-1}$) and potassium content is similar, with the exception of MG5b, which is considerably higher at 147 mg kg^{-1} .

2.1.6 Soil Acidification

The chemistry of the soils underlying the grasslands will affect the degree of acidification. Different soils have different buffer capacities affected by reaction of acids with calcium and magnesium carbonates, cation exchange, proton absorption by clay minerals and aluminium ions, humus, and dehydrated aluminium and iron oxides and solubility of soil minerals (Wild, 1993). It is likely that soils under MG5 grasslands will be better buffered than those found under U4 grasslands because they are neutral clay soils, making them less likely to respond to acid deposition. Despite this, changes in soil chemistry have been observed as a result of acid deposition in MG5 grassland. In the untreated plots of the Park Grass experiment at Rothamsted Research Station, the pH was found to decline with time in both the surface soil and the subsoils below 23 cm as a result of acid deposition (Johnston *et al.*, 1986) (figure 2.8).

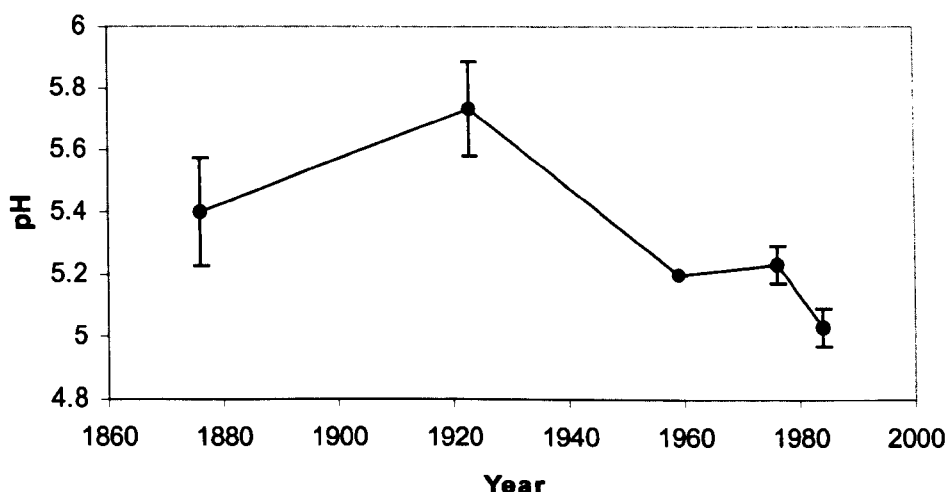


Figure 2.8 The pH of soil samples taken at various times from the surface horizon of the Park Grass Experiment (Johnston *et al.*, 1986).

Unmanured plots showed slight acidification between 1876 and 1984. There was a slight but not significant ($t = -2.5$, $p = 0.07$) increase in pH between 1876 and 1923 but since then there has been a slow decrease in pH ($t = 7.4$, $p = 0.002$) (calculated using data from Johnston *et al.*, 1986). The increasing acidity of the soils resulted in mobilisation, leaching and re-absorption of base cations through the soil and increased exchangeable aluminium (Blake *et al.*, 1999). These findings show that acidification can alter the soil chemistry of soils found under MG5 grassland.

2.1.7 Vegetation Sensitivity to Acidification

U4 grassland is an acid grassland community and as a consequence of this the vegetation is unlikely to react strongly to further acidification. The species present are already those adapted to acid soils. Mesotrophic or 'neutral' grasslands generally have a higher pH so vegetation may be more sensitive to changes in pH. There are clearly more species with low Ellenberg R scores for reaction (soil pH) in U4 grassland, which represents indicators of acid environments (figures 2.9 and 2.10).

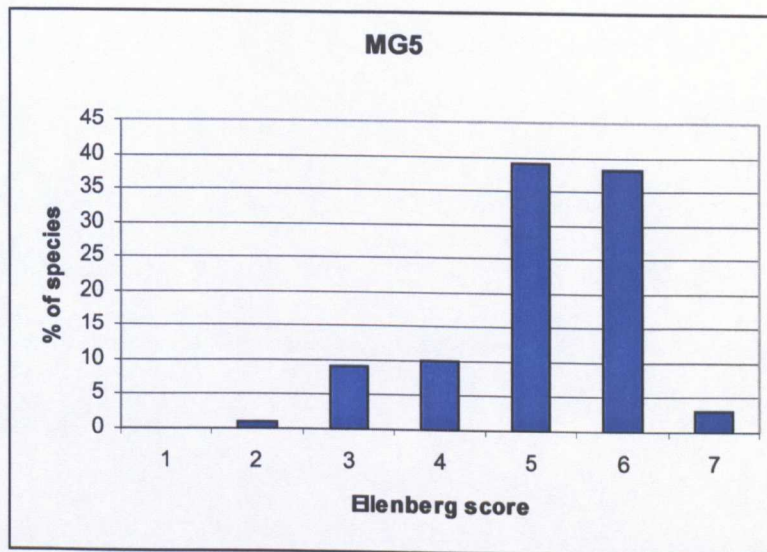


Figure 2.9 Distribution of Ellenberg R values – MG5

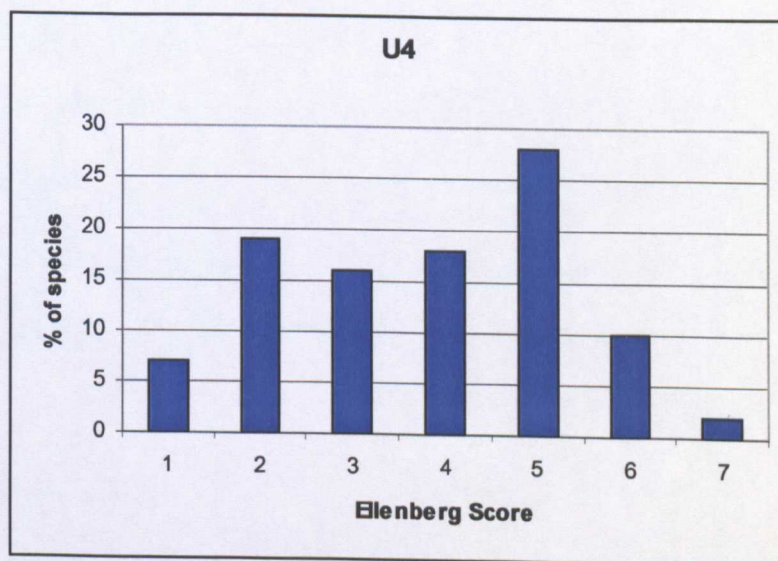


Figure 2.10 Distribution of Ellenberg R values - U4

There are a number of other effects of acidification on soil chemistry that affect plants. Aluminium, manganese, chromium, copper, nickel and zinc ions are all found at increased concentrations. The uptake by plants of phosphate and molybdate is reduced, nitrification is inhibited and nitrogen fixation by legumes is reduced unless the *Rhizobium* strain is acid tolerant (Wild, 1993).

Diekman and Dupre (1997) suggest that acidification may cause a decline in some sensitive species when no other species are available to replace them in environments that are already acidic. They used weighted average Ellenberg scores for reaction and nitrogen to assess changes in time for a number of forest sites in north-west Germany. They found that in some cases it was not possible to distinguish between the effects of acidification and eutrophication.

With the effects of acidification it is possible that there would be a change in the community. It is unlikely that U4 would change to a different community as it is the most acidic type of grassland in the UK although, importantly, the species balance may alter. Any 'new' community, not currently described in the NVC, would still be similar enough in its composition of acid-loving species to be identified as U4. MG5 grasslands could show changes towards different grassland communities including U4 grassland, although as the soils are well buffered this is unlikely.

2.1.8. Conclusions

Due to the distribution, hydrological variability and management variability it is clear that the choice of community for investigation is between MG5 *Cynosurus cristatus-Centaurea-Centaurea nigra* grassland and U4 *Festuca ovina-Agrostis capillaris-Galium saxatile* grassland. Both of these communities have characteristics making them suitable for the study. U4 grassland is the community most likely to be sensitive to eutrophication as it has the lowest Ellenberg scores for nitrogen (N) and poorly buffered soils. High nitrogen deposition is unlikely to cause a change from U4 grassland to a different community due to the acid nature of the environment, although it is likely that there would be changes in the balance of species. In MG5

grassland any changes are likely to be clearer cut with the possibility of a change toward the MG1 *Arrhenatherum elatius* grassland in addition to changes in the balance of typical MG5 species to favour those which are more nitrogen-tolerant.

In order for the reaction to nitrogen deposition to be detectable over and above other differences between sites, it is important that other differences are kept as constant as possible. The soils of U4 grasslands are more variable than for MG5 but the soils of MG5 are likely to be better buffered against the effects of acidification.

MG5 grasslands are of great conservation importance in the UK and if they are impacted by nitrogen deposition this would be a very important finding. However, the very importance of these grasslands creates practical problems. The majority of MG5 grasslands are protected for conservation and it would not always be possible to remove soils and plant material.

There are a number of important factors that must also be considered in the selection of individual sites, including the variability in altitude, climate, soil pH, drainage and localised ammonia deposition. Homogeneity is important both within and between sites.

In conclusion the probable sensitivity of plants and soils to eutrophication and the low buffering capacity of the soils of the U4 community are the most important factors making it the best choice of community for this study.

2.2. Site selection

Using information supplied by English Nature (EN), Countryside Council for Wales (CCW) and Scottish Natural Heritage (SNH) a database of all recorded U4 grasslands in the British Isles was compiled. This consisted primarily of protected areas including Sites of Special Scientific Interest (SSSI), National Nature Reserves (NNR) and National Parks. Sites under five hectares in extent were removed on the basis that they were unlikely to supply a sufficient area of U4 grassland for the study. This information was entered into the Geographical Information System package ArcInfo. Due to the large number of sites to be surveyed over a short time period, it was important not to waste time in reaching very remote sites. Hence sites were buffered with a circle representing the site area and a further 500m radius. This was then overlaid with the road network and sites that did not intersect a public road were removed. A map of total nitrogen deposition was then overlaid to allow a nitrogen deposition value to be assigned to each of the potential field sites. The sites were divided according to four bands of nitrogen deposition: 0-10 kilograms of nitrogen per hectare per year; 10-20 kg N ha⁻¹ yr⁻¹; 20-40 kg N ha⁻¹ yr⁻¹ and 40-60 kg N ha⁻¹ yr⁻¹. Sixty sites were randomly selected, five in the 0-10 kg N ha⁻¹ yr⁻¹ band, 15 in 10-20 kg N ha⁻¹ yr⁻¹, 20 in 20-40 kg N ha⁻¹ yr⁻¹ and 20 in 40-60 kg N ha⁻¹ yr⁻¹.

Permission to gain access to all sites was sought through the respective nature conservancy bodies and all landowners were contacted. If it was not possible to gain access to a selected site the nearest appropriate site was used as a replacement.

A further 8 sites were visited between June and August 2003. These were chosen within the range of $7\text{--}13\text{ kg N ha}^{-1}\text{ yr}^{-1}$ to fill a gap in the previous year's data. These sites were chosen by contacting SNH, who suggested sites within the area identified with the correct N deposition. The island of Lundy was also targeted as it has very low N inputs and provides the lowest N deposition in the south of England. Those sites included within the study in 2002 and 2003 are portrayed in figure 2.11.

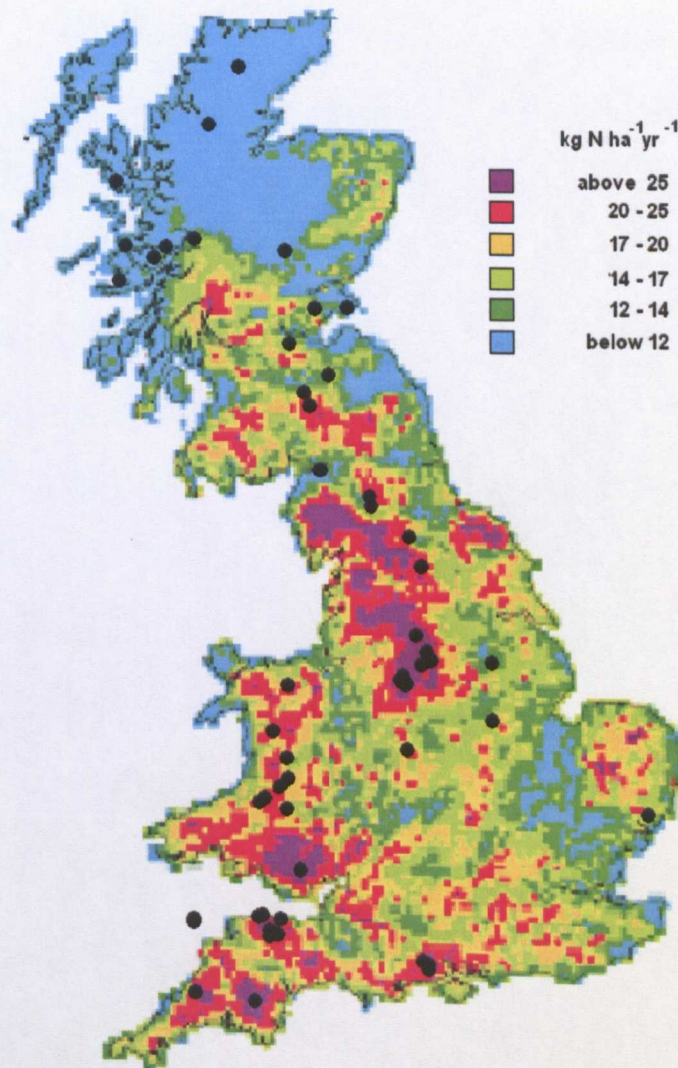


Figure 2.11 Total nitrogen deposition ($\text{kg N ha}^{-1}\text{ yr}^{-1}$) to the UK accounting for land cover (NEGTA, 2001) with field site locations shown (•).

2.3. Methods

A total of 9 field trips were made in 2002 between the beginning of May and the end of August to visit the 60 field sites (grid references and site names are provided in appendix 2). A standard sampling protocol was applied to each site.

A 100 x 100 metre area was selected for the survey; this area was always more than 50m from roads, more than 2m from any field or compartment boundaries and contained at least 50% U4 grassland. A brief survey of the sampling area was made in order to exclude any areas significantly different from a U4 community such as furrows, paths and animal feeding or latrine areas. This process was carried out in order to eliminate the effects of stock movement and water table differences caused by ditches and shaded areas. A random number table was used to select 5 points for survey within the remaining areas of U4 grassland. At each of these points vegetation was recorded in a 2 x 2m quadrat. Vegetation was identified to a species level and percentage cover estimated by eye. Topsoil samples were taken in two opposing corners of the quadrat. The topsoil sample was taken from the A horizon or, in cases where there was no A horizon apparent near to the surface, the O horizon was sampled. Sampling was done using a spade to lift a square of the soil and a trowel to sample from the appropriate horizon. A subsoil sample was taken as close to the centre of the quadrat as possible at 30-40cm depth using a 5cm diameter Dutch auger. 5cm diameter soil cores were also taken close to the centre of the first three quadrats for measurement of bulk density at a depth of 0-10cm. All samples were stored in labelled plastic bags and kept cool in ice boxes for the duration of the field trip.

Approximately 10g leaf and stem samples of three species (*Agrostis capillaris*, *Galium saxatile* and *Rhytidiadelphus squarrosus*) were collected from the field sites. Where it was not possible to collect *Rhytidiadelphus squarrosus*, *Hypnum cupressiforme* was collected instead. These were collected from both within and in the vicinity of the quadrats. Plant samples were washed in distilled water, patted dry and stored in envelopes at the end of each day.

For each of the sites visited, a full description was made of the site including grid reference and altitude at the south-east corner of the 1 hectare square and aspect determined using a global positioning system (GPS). An estimate of slope was made. Data on mean annual rainfall, actual evapotranspiration, potential evapotranspiration and mean monthly maximum soil moisture deficit were obtained from the MORECS database (Thompson *et al.*, 1981). Temperature data averaged for the years 1971-2000 were obtained from the UK Meteorological Office (MET Office, 2002). Grazing was estimated on a scale of 1 to 3 where 1 was high intensity and 3 low intensity, and assessed based on evidence from vegetation height and amount of animal faecal material. Whether a site was enclosed (by fences, walls or hedges) was also noted.

Modelled values for nitrogen and sulphur deposition were provided for each of the sites by the Centre for Ecology and Hydrology (CEH), Edinburgh. Estimates were provided at a 5 × 5 km scale for deposition to grassland. The model is focussed on UK conditions using climate, altitude and pollution concentration data to derive values (Smith *et al.*, 2000).

3. Relationships between Nitrogen Deposition and Plant Community Structure

3.1. Introduction

This chapter focuses on elucidating relationships between N deposition and the plant community structure of U4 grasslands in the UK. Changes in species richness, diversity and National Vegetation Classification sub-community are examined together with a comprehensive look at the individual species that are affected by N deposition.

Acid grasslands in the UK are under continual threat due to either agricultural intensification or poor management. They remain, however, an important resource for agriculture, conservation and recreation (Haines-Young *et al.*, 2000). A decline in the diversity of infertile grasslands has been observed in the UK (DEFRA, 2003), which may potentially be due to N deposition.

Parts of this chapter are published as Stevens *et al.*, 2004.

3.2. Methods

Field methods are described in section 2.3

3.2.1. Community analysis

For the purposes of this project, species richness was defined as the mean number of species per quadrat. Diversity was calculated using the Shannon diversity index (H') (Kent and Coker, 1992), which accounts for both abundance and evenness (a comparison between the actual diversity and maximum possible diversity) of the species present.

$$H' = \sum_{i=1}^k p_i \ln p_i$$

where: k is the total number of species

p is the proportion of cover represented by the i th species

Shannon was selected over the more commonly used Simpson index because the later places emphasis on scarce species (Mouillot and Lepretre, 1999). There are many scarce species within the dataset, but these are not the focus of the study. Objective confirmation of the grasslands as the community U4 was made using the computer programme MATCH version 2 (Unit of Vegetation Science, University of Lancaster).

3.2.2. Regression analysis

Regression analysis allows relationships to be determined between a dependent variable and 1 or more independent variables. It quantifies the amount of variation in

the dependent variable explained by the independent variable. Using a stepwise multiple regressions allows the identification of subset models where the number of independent variables is large. Significant variables are entered into the regression equation ordered according to the amount of variability they explain. In the output, the first variable explains more of the variation in the dataset than do later variables, which explain additional variation. Variables explaining the same variation in the dataset as a more important variable are not included in the model.

Stepwise multiple regression was used to create the models between measures of biodiversity and potential environmental drivers. Multiple regression assumes that the relationship between the X (independent) and Y (dependent) variables is linear, the scale of the variability of the errors is constant at all values of X, and the errors are independent and normally distributed (Bowman and Robinson, 1990). These assumptions were examined using residual plots and no violations were found (appendix 3). I also tested if any outlier points skewed the regression relationships by successively removing the largest outliers and re-running the stepwise regressions. Variance inflation factors were examined for all of the regressions. Variance inflation factors measure the degree of multi-collinearity or interdependence between independent variables.

3.2.3. Canonical Correspondence Analysis

Canonical correspondence analysis (CCA) is a multivariate ordination technique for direct gradient analysis. Species composition is directly related to measured environmental variables (Palmer, 2002). It assumes species have unimodal distributions along environmental gradients. The resultant ordination diagram

conveys large amounts of information regarding the environmental variables and their relations to sites and species. The association of different environmental variables can be estimated by the angle of incidence of the lines describing the variables, with narrow angles describing environmental variables that are highly positively related. CCA distributes individual species in the ordination diagram in a position that reflects their net tolerance to all of the environmental factors. Sites are located on the ordination diagram in relation to the environmental variables. The x axis on the diagram represents the axis corresponding to the greatest amount of variation in the dataset (axis 1) and the y axis (axis 2) the next greatest amount of variability. The importance of any environmental variable in discriminating among different species can be estimated by forward selection; this is represented on the diagram by the length of the arrow. Forward selection uses a Monte Carlo permutation test to assess the usefulness of each environmental variable in the ordination model (Leps and Smilauer, 2003). The amount of variance explained by a variable is denoted using a LambdaA value. CCA was carried out using CANOCO 4.5 (ter Braak and Smilauer, 2002).

3.2.4 Discriminant Function Analysis

Discriminant function analysis (DFA) is used to determine the amount of variance in the dependent variable explained by the independent variable as in regression analysis. In this case, however, the dependent variable is categorical, not continuous. It is used to determine the effects of continuous variables on two or more naturally occurring groups (Sokal and Rohlf, 1995). DFA was used in this study to identify the effects of nitrogen deposition and environmental variables on the grassland sub-community.

3.3. Results

3.3.1. Sub-community

All of the grasslands examined as part of the investigation had a match of greater than 40% to a U4 (*Festuca ovina*-*Agrostis capillaris*-*Galium saxatile* grassland) sub-community. Four out of the 5 U4 sub-communities occurred in the survey, and their frequency in the sample is shown in figure 3.1. U4c (*Lathyrus montanus*-*Stachys betonica*) and U4d (*Luzula multiflora*-*Rhytidiadelphus loreus*) both have localised distributions and consequently occurred only rarely or not at all in the survey.

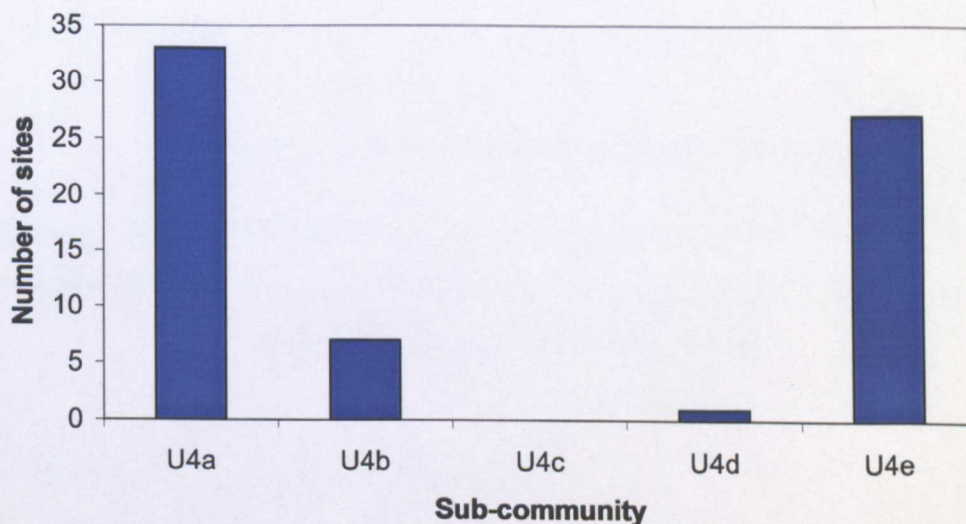


Figure 3.1 Frequency within survey of sites across NVC sub-communities.

U4a (typical sub-community) and U4b (*Holcus lanatus*-*Trifolium repens* sub-community) and U4e (*Vaccinium myrtillus*-*Deschampsia flexuosa*) occur across the gradient of nitrogen deposition (figure 3.2) although U4b does not occur in the dataset at levels of deposition greater than $27 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Among 8 variables (altitude, slope, aspect, enclosure, grazing, sulphur deposition, soil moisture deficit and total inorganic N deposition) examined, discriminant function analysis showed that total nitrogen deposition was the most important variable in determining sub-community

($f=3.71$, $p<0.05$). No other variables were significant. The data presented in figure 3.2 do not indicate a clear relationship with N deposition.

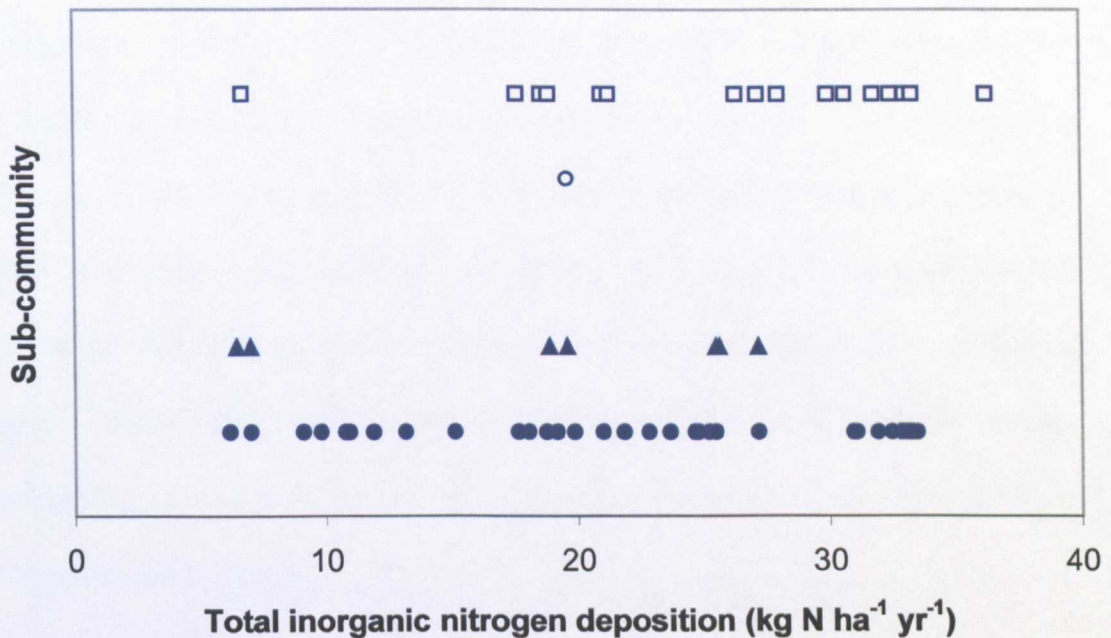


Figure 3.2 NVC sub-community in relation to total inorganic nitrogen deposition. Symbols on graph: U4a - Filled circle, U4b - filled triangle, U4d - empty circle, U4e - empty square. Some symbols overlap.

3.3.2. Canonical Correspondence Analysis

CCA shows the relative importance of the environmental variables considered (figure 3.3). Forward selection showed that total inorganic nitrogen deposition was the most important variable in relation to the species composition ($\text{Lambda}1A=0.1$). The length of the arrows in figure 3.3 represents the relative importance of the environmental variables. Total inorganic nitrogen deposition was close to the first axis of variation in the dataset, representing the greatest degree of variation. The angles of arrows representing environmental variables can be used to examine their relationship to each other. High total nitrogen deposition was strongly correlated with all of its components (i.e. NO_x , NH_4 etc.). Altitude was also highly correlated with N

deposition as at high altitudes there is the additional component of orographic deposition. As would be expected actual evapotranspiration, potential evapotranspiration, maximum soil moisture deficit and temperature were all closely correlated with each other. High evapotranspiration and soil moisture deficit were also negatively related to mean annual precipitation. With the exception of MAP these factors are all positively correlated with high N deposition, a pattern that is possibly an artefact of the majority of low deposition sites being located in the cooler north of the UK. This also applies to the slightly anomalous position of the rainfall arrow on the diagram, going in a different direction to wet deposition inputs of nitrogen. Total sulphur deposition is slightly related to nitrogen deposition, suggesting that, although they differ in their sources, there is much overlap in the incidence of high deposition.

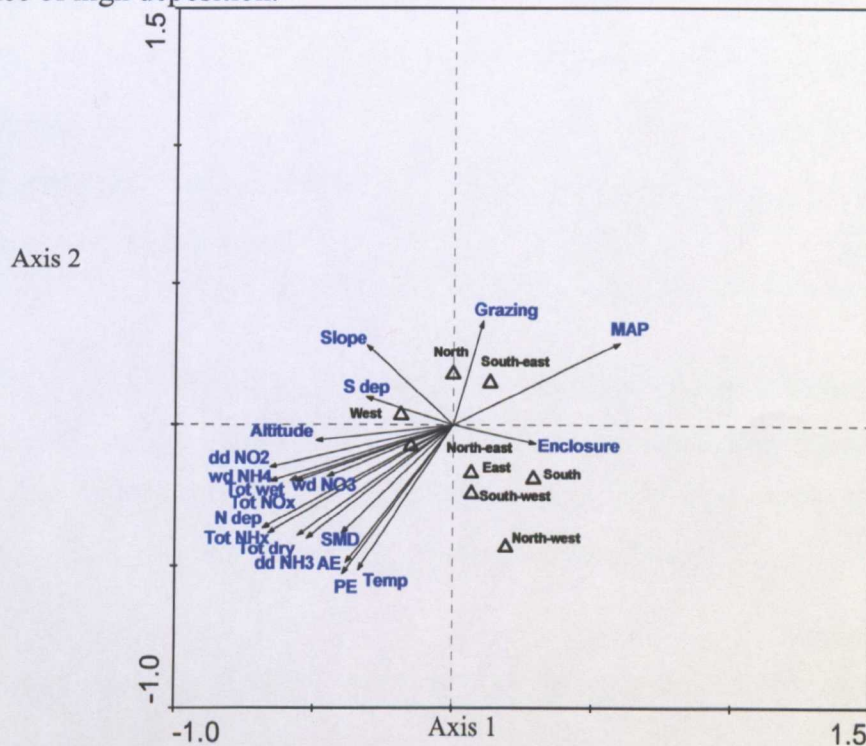


Figure 3.3 CCA ordination diagram showing environmental variables.

Abbreviations are as given in table 3.1 plus: dd – dry deposition, wd – wet deposition, Tot – total. Δ represents discrete environmental variables; \rightarrow represents continuous environmental variables with arrows pointing in the direction of increasing values.

Variable (Units) (direction/significance of correlation)	Abbreviation	Range
Total nitrogen deposition ($\text{kg N ha}^{-1} \text{ yr}^{-1}$) (-)	Ndep	6.2 – 36.3
Total deposition $\text{NH}_3 + \text{NH}_4^+$ ($\text{kg N ha}^{-1} \text{ yr}^{-1}$) (-)	N-red	2.8 – 31.2
Total deposition $\text{NO} + \text{NO}_2 + \text{NO}_3^-$ ($\text{kg N ha}^{-1} \text{ yr}^{-1}$) (-)	N-ox	2.2 – 12.6
Sulphate deposition ($\text{kg SO}_4^{2-} - \text{S ha}^{-1} \text{ yr}^{-1}$) (-)	Sdep	6.6 – 28.7
Acid deposition (total N + total S, $\text{kg ha}^{-1} \text{ yr}^{-1}$) (-)	Aciddep	13.0 – 61.4
Mean annual temperature ($^{\circ}\text{C}$) (-)	Temp	6.6 – 10.6
Mean annual precipitation (mm) (+)	MAP	594 - 3038
Mean annual actual evapotranspiration (mm) (-)	AE	35.7 – 49.3
Mean annual potential evapotranspiration (mm) (-)	PE	35.8 – 54.3
Mean annual soil moisture deficit (mm) (-)	SMD	3.4 – 51.4
Altitude (m) (-)	Altitude	15- 692
Litter cover (%) NS	Litter	0 - 24
Slope ($^{\circ}$) NS	Slope	0 - 60
Aspect ($^{\circ}$) NS	Aspect	0 - 315
Grazing intensity (visual inspection, scale 1-3) NS	Grazing	1 - 3
Enclosure (presence or absence) NS	Enclosure	Presence/absence

Table 3.1. Variables examined for relationships to plant species richness (number of species per quadrat). All variables were significantly correlated with plant species richness ($p < 0.05$), except where marked NS for not significant. + shows a positive correlation and – a negative correlation.

The number of environmental variables was reduced due to the large variance inflation factor (N dep - 1271.18) showing a large degree of multicollinearity in the CCA model produced. Total inorganic N deposition was used in place of all the highly correlated N deposition variables as it incorporates the information they convey. Soil moisture deficit was used instead of the highly correlated climatic

variables as its calculation incorporates evaporation (which in turn incorporates temperature) and rainfall. Such rationalisation of the variables gave variance inflation factors within acceptable limits.

Using the CCA ordination of the selected environmental variables produced an ordination that is easier to interpret for species and sites. Within the ordination plot (figure 3.4) sites (represented by circles) are located according to their relative species composition. Using the modelled values for N deposition at each site demonstrates that sites with high N deposition are indeed located towards the left of the diagram at the high end of the N deposition arrow and low deposition to the right of the plot. This pattern confirms that N explains a large degree of the variation between the sites. Indeed N deposition is the best discriminator between sites, with other environmental variables explaining lesser degrees of variation. The most important of these other variables are soil moisture deficit followed by (in order of descending importance) altitude, enclosure, grazing and sulphur deposition. Slope explains the least variation in the species composition between sites (figure 3.4).

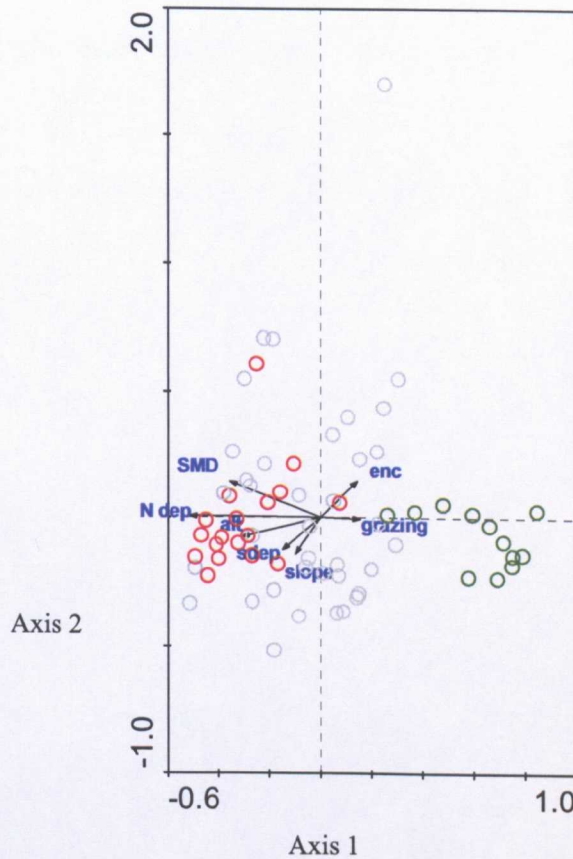


Figure 3.4 CCA ordination diagram showing selected environmental variables.

Green circles represent sites with less than $12 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ total inorganic N deposition and red over $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

Examination of the species biplot (figure 3.5) shows that the environmental variables do not explain a large degree of variation in the overall variation in species composition. However it is possible to identify species that are either negatively or positively affected by nitrogen deposition. Highest nitrogen deposition is found to the left of the ordination diagram and low deposition towards the right. Species strongly correlated with high nitrogen deposition (figure 3.5) are bilberry (*Vaccinium myrtillus*), *Hypnum cupressiforme* and carnation sedge (*Carex panicea*). Mat grass (*Nardus stricta*) and *Dicranum scoparium* also show a weaker positive correlation with N deposition. Species that were negatively correlated with nitrogen deposition were ribwort plantain (*Plantago lanceolata*), *Hylocomium splendens*, eyebright

(*Euphrasia officinalis* agg.), harebell (*Campanula rotundifolia*), heather (*Calluna vulgaris*) and to a lesser extent common birdsfoot trefoil (*Lotus corniculatus*).

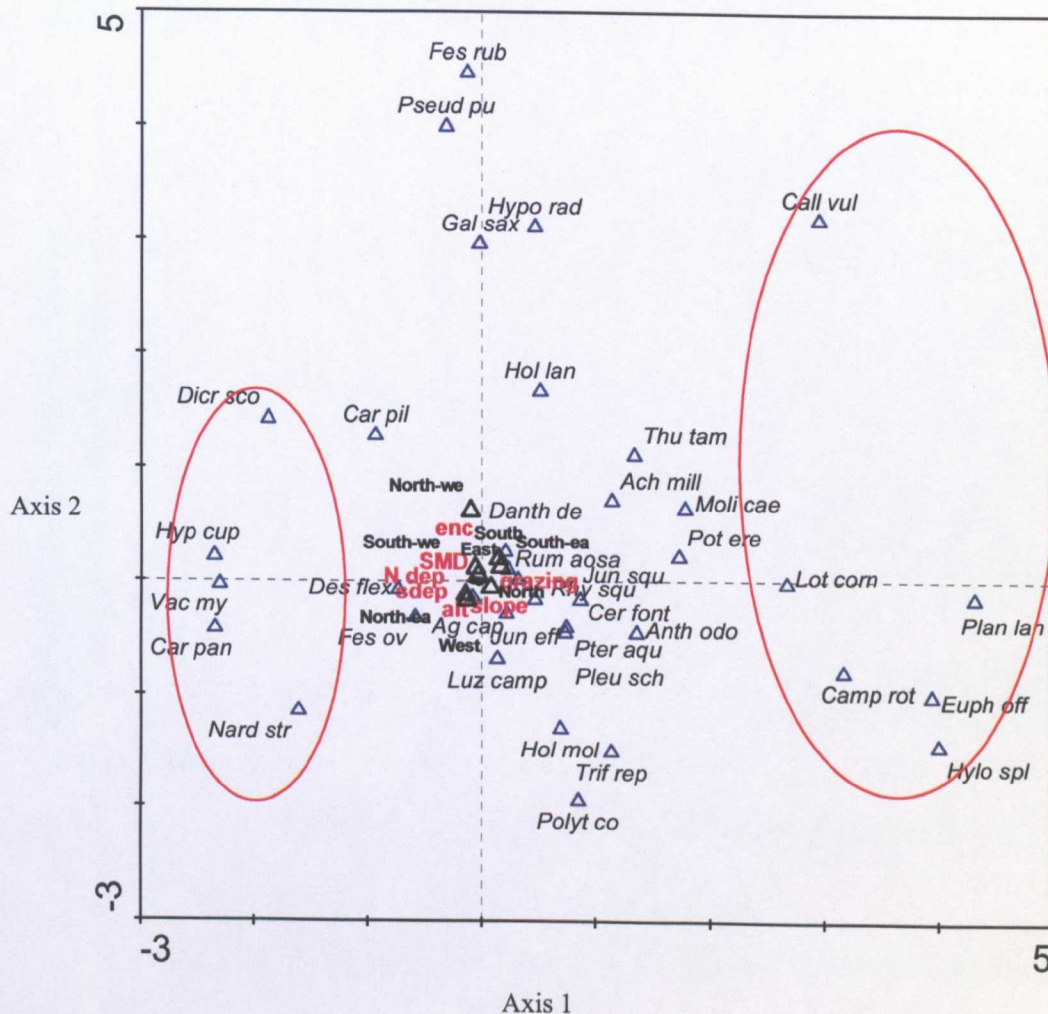


Figure 3.5 CCA ordination biplot showing species and environmental variables. N deposition lies along axis 1.

3.3.3. Species Richness

Species richness showed large variability between sites, ranging from a mean (for 5 quadrats) of 7.2 to 27.6 species per quadrat. Values are given in appendix 4. Of 16 variables measured (table 3.1) to account for the variability in local species richness, total deposition of inorganic nitrogen (*'Ndep'*, kg N ha⁻¹ y⁻¹) was the most important predictor, explaining over half of the variation in the number of species per quadrat

(Eq. 3.1). The trend is linear and negative (figure 3.6), indicating that for every 2.5 kg ha⁻¹ yr⁻¹ of inorganic N currently deposited on an acid grassland, a mean of 1 additional species is excluded from a randomly placed 4m² quadrat:

$$\text{Species richness} = 23.3 - 0.41(N_{\text{dep}}) \quad (r^2 = 0.55, p < 0.0001) \quad (\text{Eq. 3.1})$$

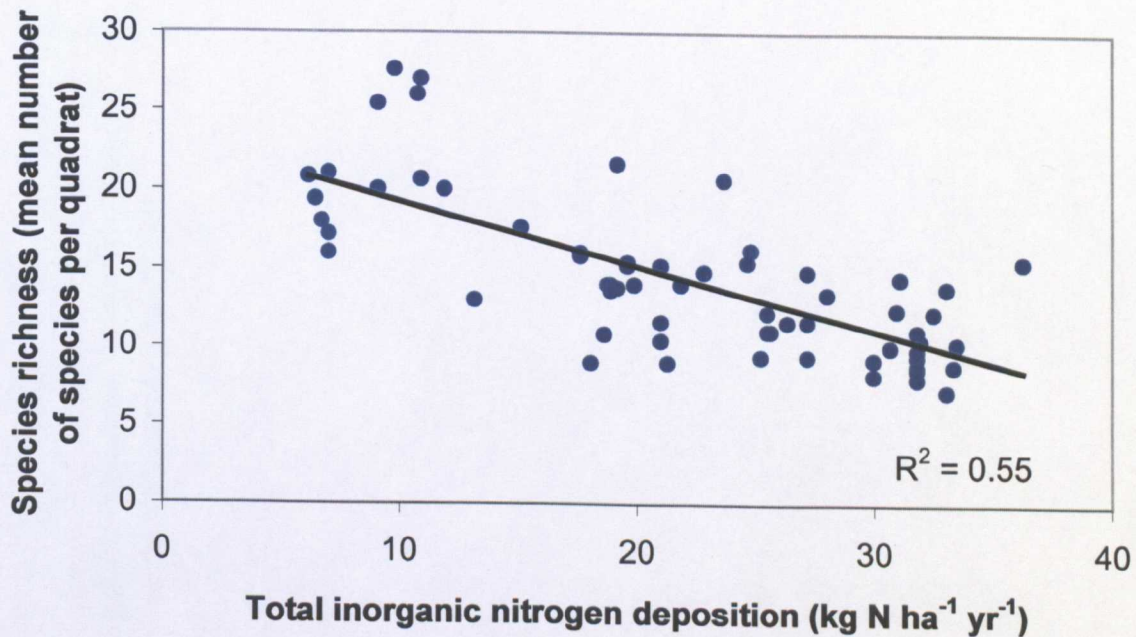


Figure 3.6 Decline in species richness with increasing N deposition.

Stepwise multiple regression showed that after accounting for nitrogen deposition, mean annual precipitation ('MAP', mm) explained an additional 8% of variability in species richness. A further 3% was explained by altitude ('Alt', m). In total, over 65% of the variability in local species richness could be explained by these three variables:

$$\text{Species richness} = 17.8 - 0.16(N_{\text{dep}}) + 0.03(\text{MAP}) - 0.007(\text{Alt})$$

$$(R^2 = 0.65, p < 0.001) \quad (\text{Eq. 3.2})$$

Variance inflation factors were found to be within acceptable limits ($VIF = 2.9$).

Examination of linear regressions for total NO_x and NH_x deposition against species richness shows that the decline in species richness is mainly accounted for by NH_x deposition ($r^2=0.54$, $p<0.001$) rather than NO_x deposition ($r^2=0.29$, $p<0.001$) (figures 3.7 and 3.8). NO_x deposition appears to show that species richness falls steeply, levelling off at $5.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

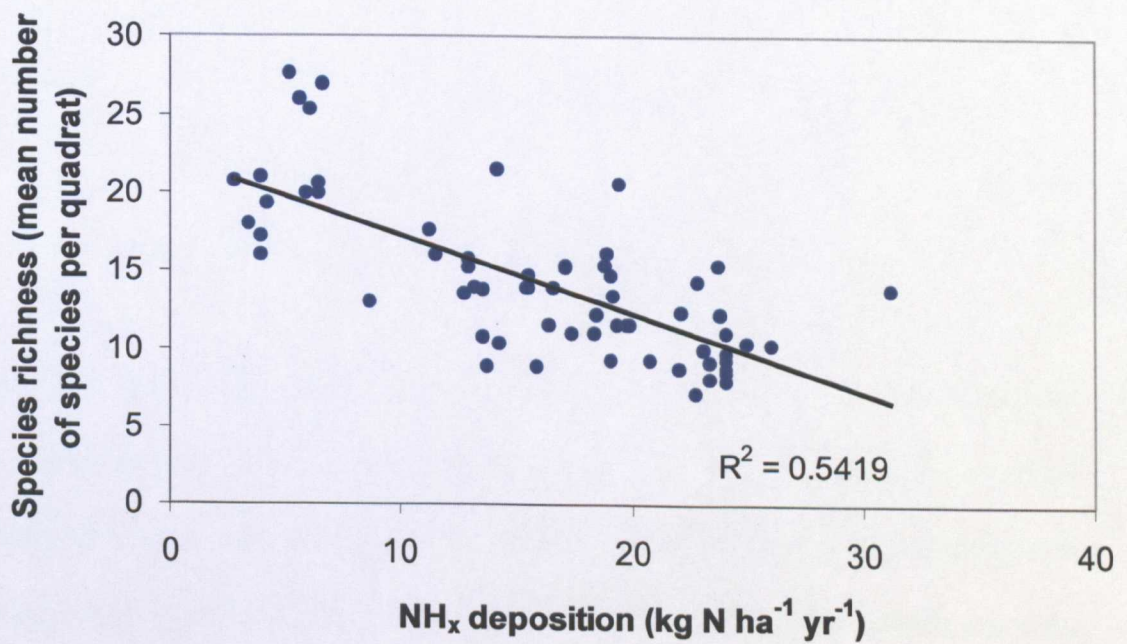


Figure 3.7 Declining species richness with NH_x deposition.

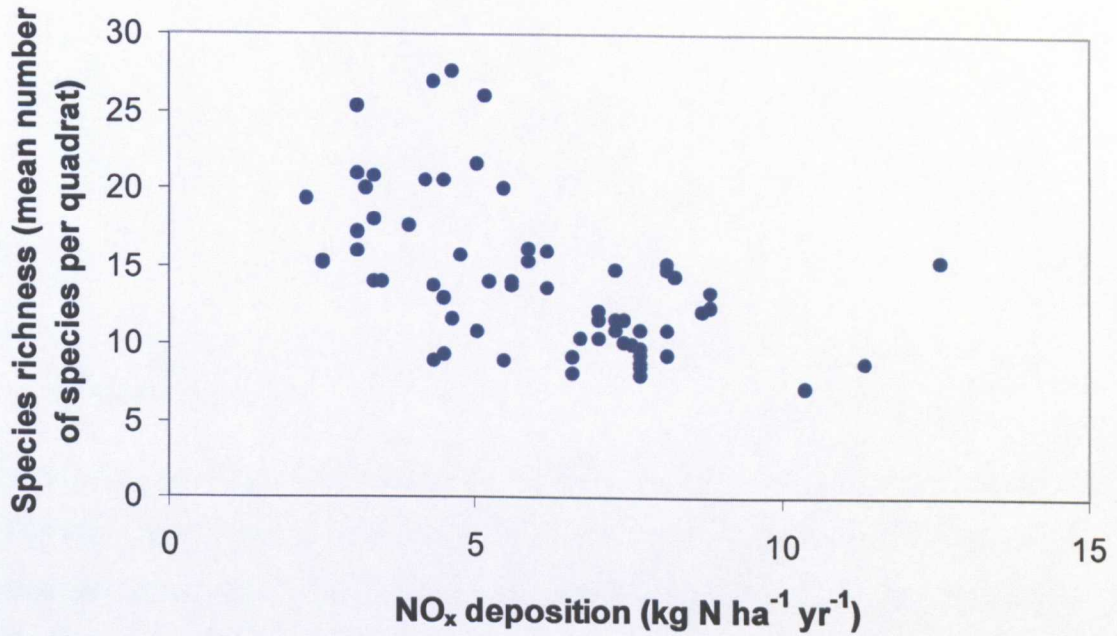
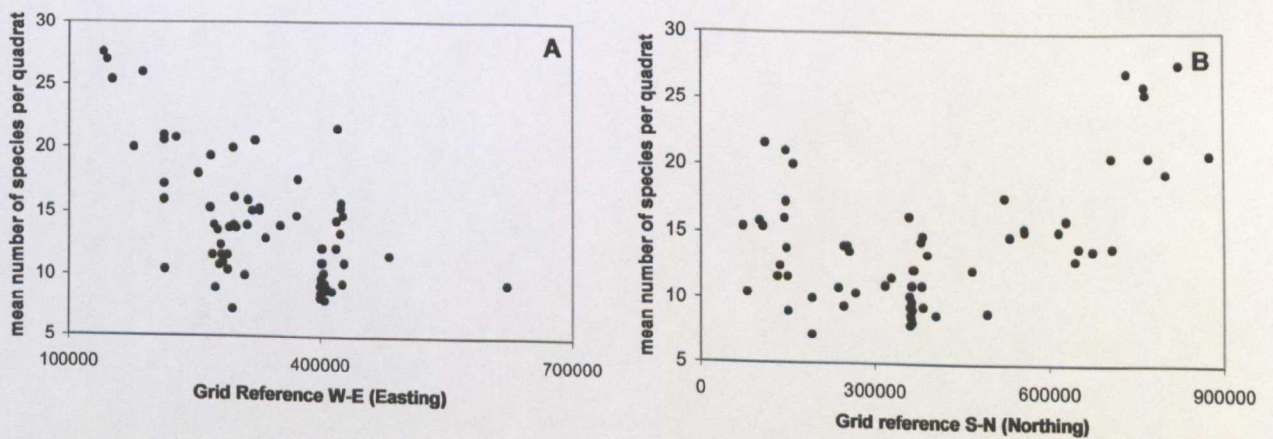


Figure 3.8 Declining species richness with NO_x deposition.

Species richness was highest in four sites in western Scotland, but overall showed high variability from west to east (figure 3.9A). There was a clearer latitudinal gradient, with some sites of high species richness in the extreme south, and a gradual increase in species richness from south to north (figure 3.9B). These trends were the reverse of trends in nitrogen deposition (figures 3.9C and 3.9D).



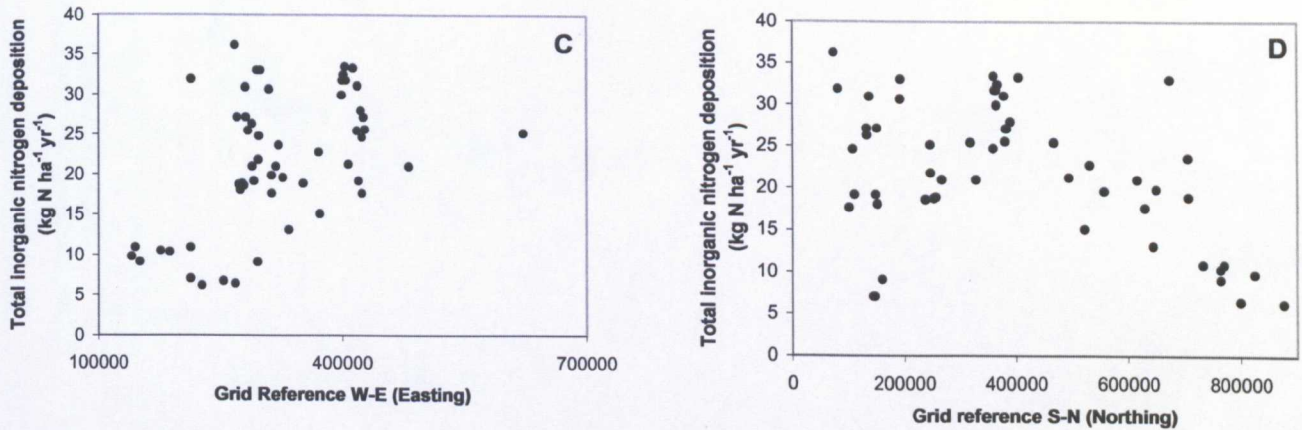


Figure 3.9A Acid grassland species richness plotted against west-east grid reference (British National Grid) (longitude range of sites: W006.2909 - E001.2497). **B.** Species richness versus south-north grid reference (latitude range of sites: N50.8119- N58.2294). **C.** Total inorganic N deposition plotted against west-east grid reference. **D.** Total inorganic N deposition versus south-north grid reference.

The greater part of the decline in species richness is accounted for by a loss of forb cover and richness (figs 3.10A and 3.10B). Both richness and cover decline linearly with increased N deposition (cover - $r^2=0.32$, $p<0.01$; richness - $r^2=0.47$, $p<0.01$). Conversely, although grass species richness declines with increasing N deposition (figure 3.12A) ($r^2=0.16$, $p<0.01$) grass cover shows an increasing trend, although this is not significant (figure 3.12B) ($r^2=0.05$, $p<0.08$). There is no significant relationship between N deposition and moss cover or richness (figures 3.12A and B).

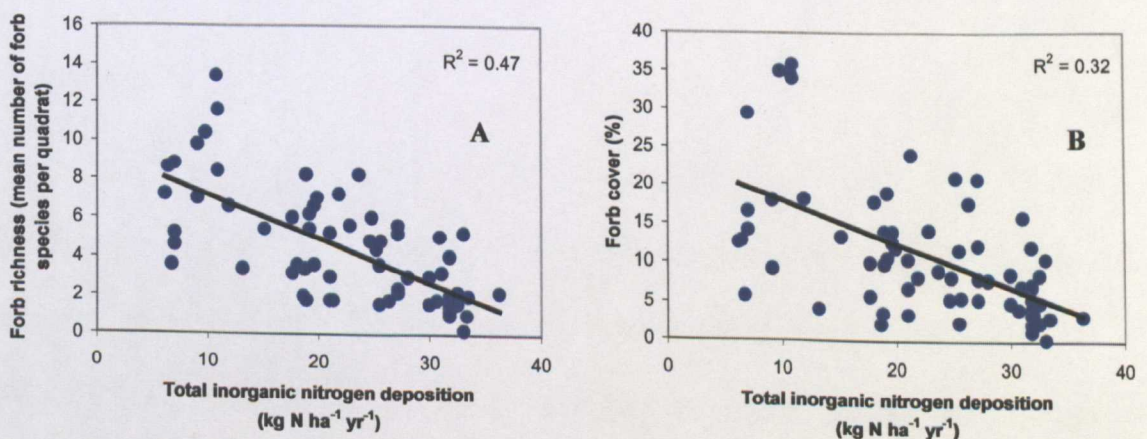


Figure 3.10 Forb richness (A) and cover (B) versus N deposition.

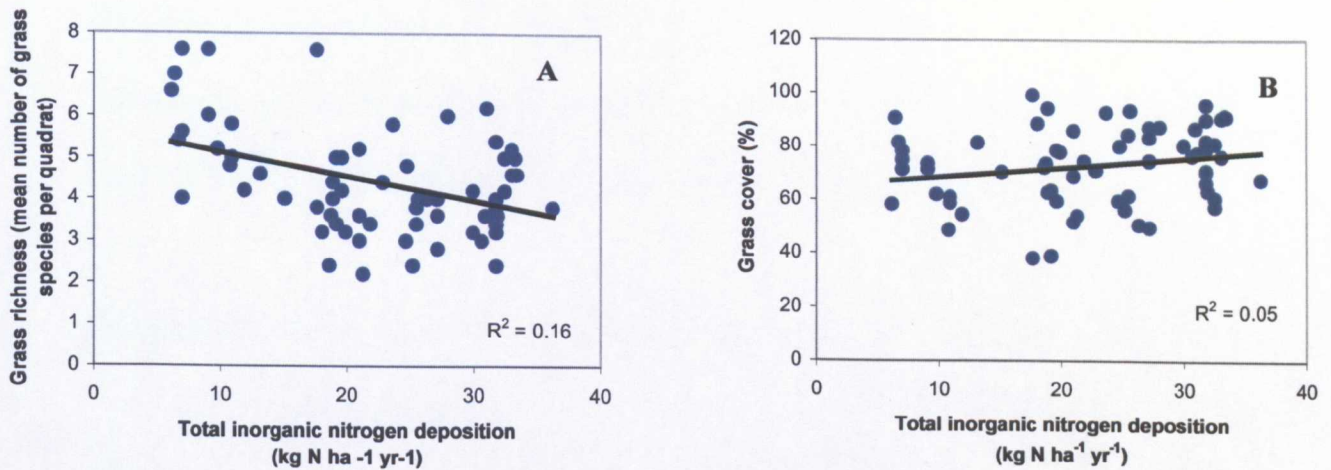


Figure 3.11 Grass richness (A) and cover (B) versus N deposition.

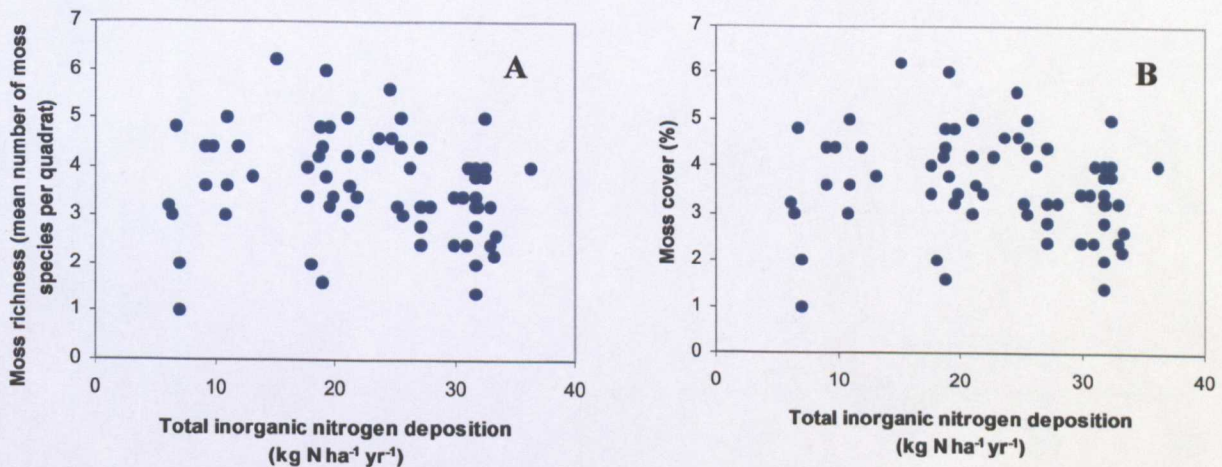


Figure 3.12 Moss richness (A) and cover (B) versus N deposition.

3.3.4. Diversity

Diversity, measured by the Shannon diversity index (H'), followed a very similar pattern to species richness. Stepwise multiple regression showed that total deposition of ammonia plus ammonium was the most important environmental variable of the 16 examined (table 3.1) in influencing diversity. Figure 3.13 shows the linear regression of diversity and total NH_x deposition (Eq. 3.3 $r^2=0.42$, $p<0.001$). A further 11% of the variation was explained by mean annual rainfall (MAP , mm), and grazing intensity

(*Grazing*, 1-3) an additional 3% (Eq. 3.4). The model explained a total of 57% of the variation in species diversity. Variance inflation factors were again within prescribed limits ($VIF = 2.5$). NO_x deposition (figure 3.13) explains less of the variation.

$$\text{Shannon diversity index} = 0.98 - 0.01(\text{NH}_x) \quad (\text{Eq. 3.3})$$

$$\text{Shannon diversity index} = 6.72 - 0.009(\text{NH}_x) + 0.001(\text{MAP}) + 0.40(\text{Grazing}) \quad (\text{Eq. 3.4})$$

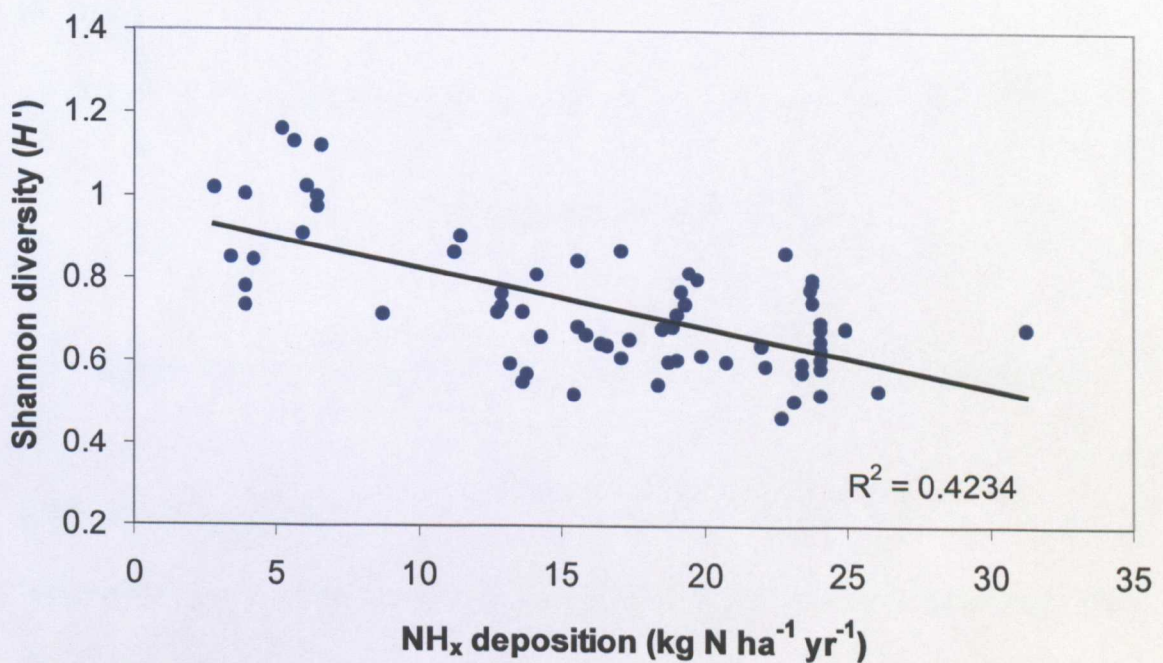


Figure 3.13 Decline in Shannon diversity with increasing NH_x deposition.

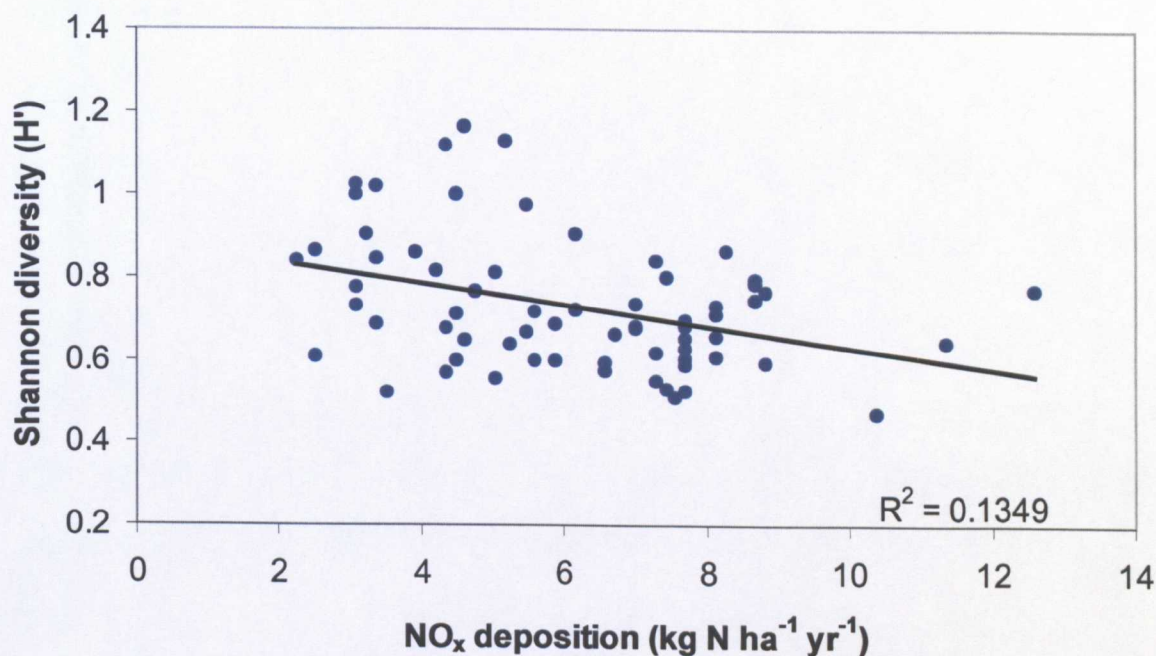


Figure 3.14 Decline in Shannon diversity with increasing NO_x deposition.

3.3.5. Ellenberg Scores

Neither the original mean Ellenberg scores for nutrient status (N) (Ellenberg, 1979), nor those modified for use in the UK (Hill *et al.*, 1999) were significantly related to the level of N deposition on the sites (figure 3.16). A weak but significant relationship was found between Ellenberg scores for pH (R – reaction) and N deposition ($r^2 = 0.07$, $p < 0.05$). Further graphs showing that there is no significant relationship between N deposition and Ellenberg scores are given in appendix 5.

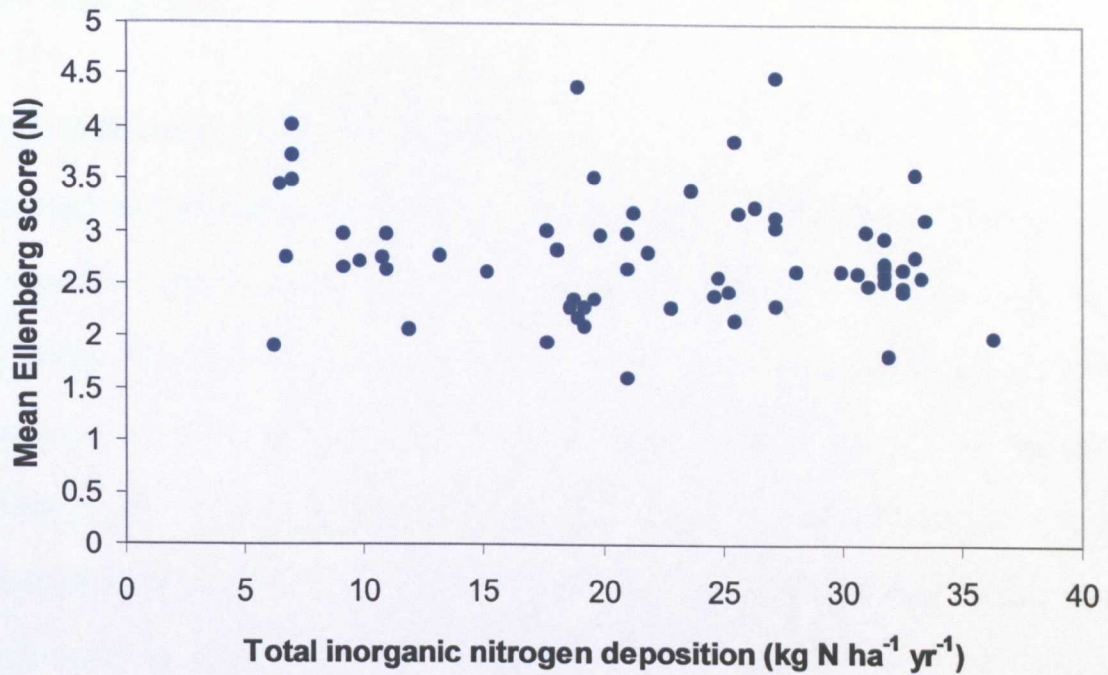


Figure 3.15 Mean Weighted Ellenberg Score (N) versus N deposition.

3.3.6 Other Environmental Variables Correlated to Species Richness

Other environmental variables correlated with species richness, which are not included in the outcome of the multiple regression (precipitation and altitude are included), were: mean annual potential evapotranspiration ($r^2 = 0.26$, $p < 0.01$), mean annual maximum soil moisture deficit ($r^2 = 0.15$, $p < 0.01$), and total sulphur deposition ($r^2 = 0.09$, $p < 0.05$). The correlation with potential evapotranspiration and soil moisture deficit may be as a result of drought reducing productivity and hence promoting species richness. Sulphur deposition may have an influence over species richness, though this may simply reflect a correlation with N deposition.

3.4. Discussion

3.4.1. Canonical correspondence analysis

Although nitrogen deposition is clearly an important variable influencing the species composition, other factors contribute. Strongly correlated with axis 1 of the ordination (figure 3.5) nitrogen deposition is the main explanatory factor of the variation within the dataset, and this relationship is confirmed by the forward selection. The arrow is very short on the ordination diagram (located on figure 3.5 by 'N dep'), indicating that it is not explaining a large portion of variation. This could indicate that there are many variables contributing to the variation.

Where a variable (e.g. a species) shows a negative correlation in the CCA species ordination diagram, this indicates a reduced occurrence and/or abundance at high N deposition (figure 3.5). A negative correlation with nitrogen deposition suggests that a species is in some way sensitive to the effects of N deposition, since all species considered occurred in over 20% of the sites and have a national distribution. It is possible that N deposition is a surrogate for another controlling variable. To investigate this individual species were examined in more detail. Species showing a negative trend with N deposition include *Calluna vulgaris*, *Lotus corniculatus*, *Plantago lanceolata*, *Campanula rotundifolia*, *Euphrasia officinalis* and *Hylocomium splendens*. *Dicranum scoparium*, *Hypnum cupressiforme*, *Vaccinium myrtillus*, *Carex panicea* and *Nardus stricta* all show a positive trend with N deposition.

The species that showed the strongest negative tendency with nitrogen deposition was *Plantago lanceolata* (figure 3.5). *P. lanceolata* is a stress-tolerant species (Grime,

1988) and is very common in U4 grassland. N addition experiments at Tadham Moor, Somerset, on an unimproved hay meadow, showed a significant decline in cover (%) of *P. lanceolata* with low N additions ($25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (Mountford *et al.*, 1993; Mountford *et al.*, 1994; Kirkham *et al.*, 1996). In ungrazed vegetation it is possible that the rosette growth form means *P. lanceolata* is unable to compete well for light with more nitrogen-demanding species. U4 grasslands, however, can be intensively grazed and competition for light is less of an issue in a short sward, suggesting that competition is either for nutrients or space at ground level. As *P. lanceolata* is a stress tolerator, the latter explanation is more likely.

The moss *Hylocomium splendens* is the next most sensitive species. *H. splendens* has been shown to decrease with nitrogen additions in several forest experiments. Doses of $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ caused a strong decline of *H. splendens* abundance in Sweden (Dirkse and Martakis, 1992) and a decline has been identified in coniferous forests in southern Germany over a 20-40 year period. The latter was attributed to sensitivity to acidification (Rodenkirchen, 1992). It is very likely that these same effects have been observed in forests are here being demonstrated in acid grasslands.

Euphrasia officinalis is also negatively correlated with high N deposition. *E. officinalis* is actually an aggregate of a number of micro-species that were not differentiated in this study. It is a small hemiparasitic annual on the roots of herbs and small shrubs (Preston *et al.*, 2002), and hence a decline in the host plants might be an explanation for the decline of *E. officinalis*. Forbs decline in cover and richness with N deposition (figure 3.11A) and there is a positive relationship between forb

cover and *E. officinalis* cover ($r^2 = 0.3$, $p < 0.01$). Frequency of *E. officinalis* also increases with increasing forb cover (figure 3.16).

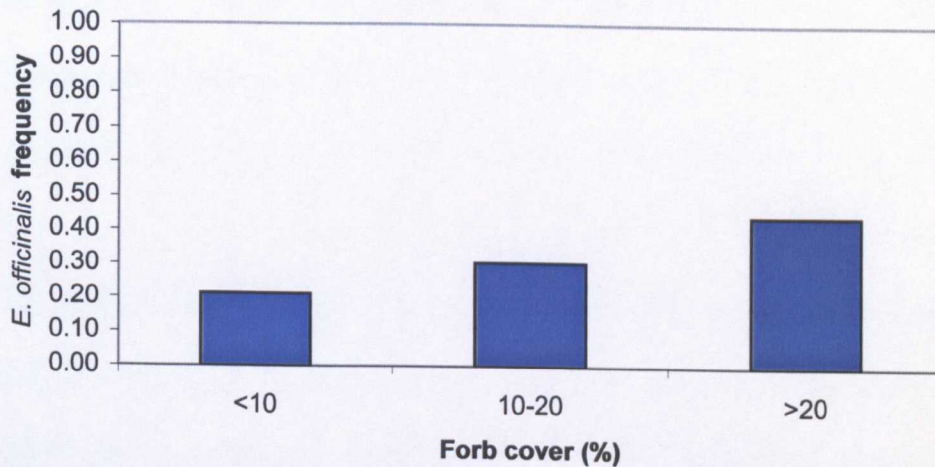


Figure 3.16 *E. officinalis* frequency versus forb cover.

Campanula rotundifolia is a species of infertile habitats (Preston *et al.*, 2002; Hill *et al.*, 1999) and is described as intolerant of competition with vigorous grasses (Sinker *et al.*, 1985). Many of grass species have been shown to increase with N addition in experimental work (e.g. Mountford *et al.*, 1993; Heil and Diemont, 1983). The present study identifies a decrease in species richness combined with little change in cover for grasses, suggesting that a few grass species are becoming dominant (figures 3.12A and B), possibly leading to the decline in *C. rotundifolia*.

Calluna vulgaris also shows a negative relation to N deposition. *C. vulgaris* is perhaps one of the best investigated species with regard to N deposition (see section 1.1.2). N addition experiments have identified a reduction in *C. vulgaris* cover as a result of competition with grasses (e.g. Heil and Diemont, 1983), increased frost sensitivity (Caporn *et al.*, 2000), winter desiccation (Sheppard and Leith, 2002) and

increased susceptibility to heather beetle (*Lochmaea suturalis*) attack (Brunsting and Heil, 1985). The sensitivity of *C. vulgaris* detected in this survey supports the hypothesis that the effects observed in N addition experiments could also actually occur at the levels of N deposited on acid grasslands in the UK. However, it is not possible to say which, if any, of the potential effects of N deposition on *C. vulgaris* is causing the decline.

The final species that shows a negative correlation with N deposition is the legume *Lotus corniculatus*. *L. corniculatus* is a species of infertile environments (Hill *et al.*, 1999), but it is tolerant of competition in grazed environments (Sinker *et al.*, 1985). It is possible that a degree of differential competitive ability is being observed in the present study *i.e.* at higher nitrogen deposition the advantage conferred by being able to fix N₂ becomes less important.

Some species sensitive to N deposition may not have been detected by this analysis because they did not occur in enough surveyed sites to allow significant trends to be demonstrated. Alternatively their distribution may already have been reduced either by N deposition or another factor.

Three species show a clear positive response to N deposition – *Hypnum cupressiforme*, *Vaccinium myrtillus* and *Carex panicea*. *H. cupressiforme* was not differentiated from *H. jutlandicum* in this study. *H. cupressiforme* is a pollution-tolerant moss (Rodenkirchen, 1992), and has been found to persist in areas where other bryophytes have declined considerably due to N deposition (Hallingback, 1992). *H. jutlandicum* has been shown to increase in cover with the addition of 20 kg N ha⁻¹

yr⁻¹ (B. J. Haworth, pers. comm.) and has shown a positive response to N addition in high Arctic heath (Gordon *et al.*, 2001). This pollution tolerance would allow *H. cupressiforme* to compete well with more sensitive mosses.

The increase in Bilberry (*V. myrtillus*) is more surprising. In Swedish forests *V. myrtillus* has been shown to be less abundant at high levels of N deposition and more susceptible to the parasitic fungal leaf pathogen *Valdensia heterodoxa* (Strengbom *et al.*, 2003). Bilberry is a species characteristic of very nutrient-poor environments (Hill *et al.*, 1999) and the increase observed in the present study may be due to the acidification caused by N deposition. *Vaccinium* spp. are among the most acid-tolerant plants (Diekmann and Dupre, 1997), and thus a decline in other species may allow them to compete more effectively, especially with the decline of the other common small shrub in U4 grasslands, *C. vulgaris*. It should be remembered that even the most nitrogen-rich sites in the study would be considered nutrient poor, which may be a reason for the difference in reaction compared to the Swedish forests.

C. panicea is also a species of nutrient-poor environments (Hill *et al.*, 1999). Although increased *C. panicea* was observed at Inverpolly National Nature Reserve between 1972 and 1989 (where other changes in species composition were attributed to increased N deposition), the greater abundance of *C. panicea* was thought to be due to reduced grazing pressure (Pitcairn *et al.*, 1991). Sedge cover has increased with experimental N additions in calcareous grasslands. This increase could possibly be attributed to a low sensitivity to P limitation (Phoenix *et al.*, 2003a).

To a lesser extent *Nardus stricta* and *Dicranum scoparium* also showed a positive correlation with N deposition. *N. stricta* is an unpalatable grass and consequently is a common invader of overgrazed grasslands and heathlands. *N. stricta* has been shown to increase shoot/tiller length, biomass and root growth in response to nitrogen additions equivalent to those from atmospheric inputs (Leith *et al.*, 1999), and also to experimental N additions in U4e grassland (Carroll *et al.*, 2003). It is thus not surprising that *N. stricta* shows a positive response in cover to N deposition in the present study. *N. stricta* has been shown to out-compete *C. vulgaris* under fertiliser additions (Hartley and Amos, 1999), suggesting that it may be one of the grasses whose vigorous growth negatively affects *Calluna*. In the present dataset where *N. stricta* is increasing, *C. vulgaris* is decreasing (figure 3.17). This trend cannot be attributed to competition without further investigation, although the findings of Hartley and Amos suggest that N deposition might combine with other factors such as grazing to alter the dynamic interaction between these two species.

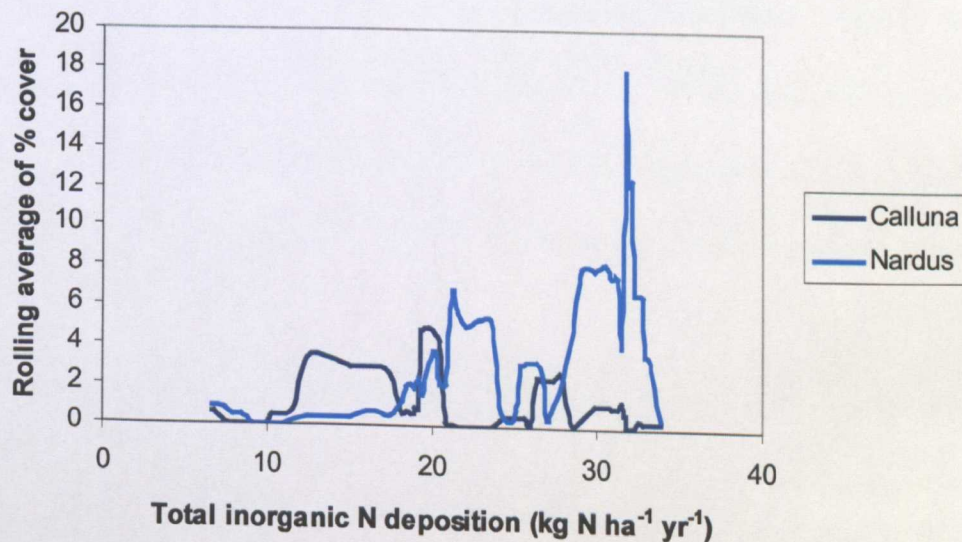


Figure 3.17 Distribution of *N. stricta* and *C. vulgaris* at different levels of N deposition.

Low levels of N addition were shown to have a slight negative effect on *D. scoparium* cover in arctic tundra (Gordon *et al.*, 2001), although this trend was not significant. Applications of 140 kg ha⁻¹ yr⁻¹ of ammonium sulphate were found to increase *D. scoparium* cover (B. J. Haworth, pers. comm.).

3.4.3. Species richness

Total inorganic N deposition is clearly an important factor in determining species richness (equation 3.1, figure 3.14). Interactions between species are complex and there are likely to be many reasons why individual species decline. However, the main reason for the decline in overall species richness is probably competition.

In the present study, equation 3.1 gives the mean species richness of an acid grassland affected by the lowest levels of N measured (5 kg N ha⁻¹ yr⁻¹) as 21.3 species per quadrat. Using this as a conservative estimate of the species richness of a pristine grassland, and substituting into Equation 3.1 the approximate mean N deposition in the eastern USA (7.7 kg N ha⁻¹ yr⁻¹ (Baumgardner *et al.*, 2002)) and central Europe (17 kg N ha⁻¹ yr⁻¹ (EMEP, 2004)), gives a current average reduction of 5.2% and 23% of acid grassland species richness respectively from the projected pristine condition.

Experimental additions of nitrogen to grasslands in the UK and North America have resulted in a loss of 3-4 % of species richness for each 100 kg N ha⁻¹ added over the course of experiments. N additions of 100 kg N ha⁻¹ yr⁻¹ resulted in approximately 30% reduction in grassland species richness over a period of 7 years in England (Mountford *et al.*, 1993), and a ca 40% reduction over 12 years in North America (Wedin and Tilman, 1996). Data from the above papers were used to give an average

annual loss of species richness for the duration of the experiments of 3.3 and 4.3% for each cumulative 100 kg N ha^{-1} deposited. Making the conservative assumption that N deposition has a fully cumulative effect on vegetation (*i.e.* no N is lost due to seepage, denitrification, etc.), the total amounts of N required to reduced species richness by 25% are respectively 757 and 568 kg N ha^{-1} for the British and American experiments.

At the mean nitrogen deposition for central Europe, the time to reach 757 kg N ha^{-1} is 44 years at a constant level of deposition, and longer if N deposition has increased from lower levels in the past, or if some N is not taken up by vegetation. The time to reach 568 kg N ha^{-1} is 33 years. This time frame is consistent with the enhanced emission of reactive N beginning around the start of the 20th century, and accelerating in its latter half (Goulding *et al.*, 1998).

The assumption that negligible N is lost by seepage is backed by experimental findings in acid grasslands, which show that even high inputs over many years do not lead to large outputs of N (Phoenix *et al.*, 2003). Although N deposition is beginning to decline in many areas of Europe (Goulding *et al.*, 1998, Wright *et al.*, 2001), the potential importance of cumulative N deposition, and the alteration of soil pH (which may never recover) may mean that a return to an 'unpolluted' condition of high species richness will not be achieved.

Standards for airborne pollutant emissions in Europe are based on the concept of "critical loads" *i.e.* 'the highest deposition of compounds that will not cause chemical changes leading to long term harmful effects on ecosystem structure and function'

(Nilsson and Grennfelt, 1988). The current critical load for nutrient nitrogen for acid and neutral grasslands in Europe has been set at $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Hall *et al.*, 1998). The present study suggests that adhering to this level as an international standard may result in a long-term degradation of grassland species richness by about 38.5% (equation 3.1). Indeed, the strong negative linear trend between nitrogen deposition and species richness in acid grasslands (figure 3.13) implies that there is no threshold for anthropogenic N deposition below which there are no effects on this community, and so the concept of a critical load has little meaning.

Forbs account for the largest proportion of species potentially lost due to N deposition (figure 3.10), and they decline in both richness and cover. Many grassland forbs tend to be low growing, and respond less rapidly to nitrogen application than grasses. Consequently forbs do not compete as strongly for light at high N deposition and are less able to take advantage of the additional N. Although grasses also showed a decline in species richness, total grass cover does not change strongly suggesting the increased dominance of a few species. These findings are consistent with research results from U4 grasslands in Derbyshire (G. Phoenix, pers. comm). The only grass species identified as responding positively to N deposition in the CCA in this study is *N. stricta* but this alone is not strong enough to explain the increased grass cover. Hence the overall increase in grass cover must be due to different species in different locations where site characteristics differ. It is surprising that bryophytes as a group do not appear to be responding negatively to N deposition as they are frequently identified as being sensitive to N inputs (e.g. Gordon *et al.*, 2001).

A decline in the diversity of infertile grasslands has been observed in the UK over the last 25 years (DEFRA, 2003). This research gives one possible explanation for the decline.

3.4.4. Species diversity

Species diversity shows a similar trend to species richness (figure 3.14) but equation 3.3 shows that the variable most closely correlated with plant species diversity is NH_x . The fact that species richness is most closely related to total inorganic N deposition whereas NH_x deposition is most closely linked with diversity would suggest that slightly different controls may be operating. The cover/abundance of individual plant species may be primarily controlled by NH_x deposition. At high deposition we see increasing dominance of a few vigorous species as they increase in cover in response to greater availability of NH_x . Carroll *et al.* (2003) relate cover to NH_4^+ application in U4e grassland in Derbyshire. Paulissen *et al.* (2004) identified differential effects of nitrate and ammonium addition on fen communities in experimental applications. The NO_x component may have additional effects on species richness that are unrelated to cover/abundance or competition. An example of an unrelated effect of NO_x and NH_x on the vegetation might be increased frost sensitivity.

As with species richness, precipitation (equation 3.4) is an important variable influencing species diversity, but altitude is replaced by grazing as a key variable in the results of the analysis. Altitude and N deposition are so closely linked in this dataset that there is an inescapable confounding of the variables. Consequently altitude is less of an influence over the diversity index because it exerts little influence over the cover of dominant species that are unrelated to N deposition. Grazing, on the

other hand, may be suppressing the cover of key dominant species (Hickman *et al.*, 2004, ten Harkel and van der Meulen, 1996) rather than species presence or absence possibly due to improved light penetration or reduced standing biomass.

3.4.5. Ellenberg scores

Mean Ellenberg scores for nutrient availability do not significantly vary in relation to nitrogen deposition. In agreement with Wilson *et al.* (1995), the present study suggests that mean Ellenberg scores are not the best method of assessing community change resulting from of nitrogen deposition. Schaffers and Sykora (2000) suggest that the N-score is not only a measure of nutrient availability, but also of other factors influencing productivity. The Ellenberg scores appear to be more effective at assessing change for some species than for others. It is also possible that the changes observed due to nitrogen deposition are too subtle to be detected by Ellenberg scores. In a study of ground flora in Swedish deciduous forests, Falkengren-Grerup and SchötteIndreier (2004) suggested the Ellenberg N values would benefit from combination with other indicators.

3.5. Summary

CCA showed that N deposition has an importance influence over vegetation species composition. Several species including *Vaccinium myrtillus*, *Hypnum cupressiforme* and *Carex panicea* showed a positive correlation with N deposition, increasing in cover/abundance. *Nardus stricta* and *Dicranum scoparium* showed a weaker positive correlation with N deposition. A number of species showed a negative correlation with N deposition, *Plantago lanceolata*, *Hylocomium splendens*, *Euphrasia*

officinalis, *Campanula rotundifolia*, *Calluna vulgaris* and *Lotus corniculatus* all have lower cover and abundance at high N deposition.

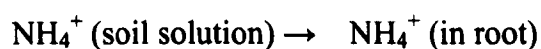
Species richness showed a negative linear trend with N deposition indicating that for every $2.5 \text{ kg ha}^{-1} \text{ yr}^{-1}$ of inorganic N currently deposited on an acid grassland, a mean of 1 additional species is excluded from a randomly placed 4m^2 quadrat. Starting from a projected pristine condition of $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, an average reduction in species richness is currently occurring of 5.2% at the mean N deposition for eastern US and 23% at the mean for central Europe. Forbs declined in both cover and abundance whilst grasses showed a slight increase in cover.

4. Relationships between Nitrogen Deposition and Soil Chemistry and Processes

4.1. Introduction

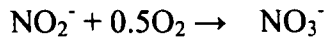
This chapter will focus on elucidating the relationships between N deposition and the soil biogeochemistry of U4 grasslands in the UK. Variability in the soil pH, total nitrogen concentration, total carbon concentration, C:N ratios, major exchangeable anions and cations, plant available phosphate, trace metals, natural abundance of ^{15}N and ammonium mineralization as a function of N deposition are examined.

N deposition has the potential to both acidify and enrich soils. Ammonium can acidify by two potential routes. When taken up by plant roots, as with other base cations (e.g. Ca^{2+} , K^+ , Mg^{2+}), ammonium releases a further H^+ in order to remain electronically neutral.

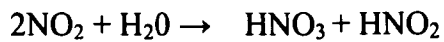


The process of nitrification, converting NH_4^+ to NO_3^- also leads to acidification if the nitrate is permanently removed from the profile by leaching, which causes an imbalance in the natural cycles in the soil (NEGTA, 2001).

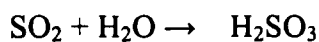




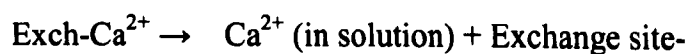
Nitrogen oxides in the atmosphere acidify by the conversion of NO_2 to NO_3^- , a process that releases an H^+ ion. This is deposited in precipitation. NO_2 gas can be deposited directly as dry deposition. This acidifies by hydrolysis occurring on the surface of the soil and the vegetation.



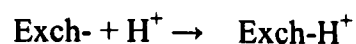
This process also occurs with dry deposited SO_2 , which can form H_2SO_4 or H_2SO_3 via one of several reactions, e.g.:



NO_3^- and SO_4^{2-} , already oxidised in the atmosphere, acidify by removing base cations from ion exchange sites in the soil – the mobile anion hypothesis (Johnson *et al.*, 1986). As the deposited anions leach through the soil they remove a cation from the ion exchange sites conserving electroneutrality. This removal leaves the exchange sites open to be occupied by H^+ ions.



(Ca^{2+} and SO_4^{2-} leach out of soil solution together)



A number of effects of N deposition on soils have been observed including the acidification of peat soils due to acid deposition *e.g.* in Scotland (Skiba *et al.*, 1989). Increasing acidity can have important consequences determining concentrations of potentially toxic metals, affecting bacteria and other microbes and causing changes in the vegetation (Falkengren-Grerup, 1995).

The second effect of nitrogen inputs is eutrophication, the enrichment of the soil solution by nitrogen. This process can affect vegetation, causing increased productivity and species changes (as discussed in chapter 1). Alternatively nitrogen can be leached through the soil profile and enter the waterways.

Much of what we know concerning the effects of eutrophication on soils relates to forests (see section 1.1.2.1), and considerably less is known with regard to grassland soils (1.1.2.4). Indicators of N enrichment have been described for forest soils including N concentration in pine needles (Dise *et al.*, 1998) and changes in the litter C:N ratio and rates of soil processes (Aber, 2003). The effects of soil N enrichment in soils have been detected in the UK and the rest of Europe in forests leading to increased amounts of nitrate in streams (Wright *et al.*, 2001). The soils of U4 grasslands are extremely variable, ranging from sands to brown earths to peats but all are acidic with a low buffering capacity and low nutrient status relative to intensively farmed grassland. Hence U4 soils are potentially sensitive to acidification and / or eutrophication via N deposition.

Changes in the soil pH, total nitrogen and carbon together with C:N ratios, anions and cations, plant available phosphate, metals, natural abundance of ^{15}N and ammonium

mineralization are examined in this chapter, giving information with regard to acidification and consequent mobilisation of metals, nitrogen enrichment and microbial process rates. These measurements will demonstrate the effect that N deposition is currently having on the soils of acid grasslands in the UK.

4.2. Methods

Five subsoil samples taken at a depth of 30-40 cm were collected from each of the 68 survey sites together with topsoil samples taken from the A horizon. The topsoil samples were taken from each corner of the quadrat and bulked to give five samples per site. Soil collection methods are fully described in section 2.3. All topsoil and subsoil samples were dried overnight in an oven at 40°C and ground to <2mm prior to analysis.

4.2.1. Soil pH

Soil samples were thoroughly mixed to ensure homogeneity. 10 g of soil was weighed out and placed into a 50 ml beaker. 10 ml of distilled water were added using a pipette and stirred for 30 minutes (Thomas, 1996). pH was determined using an Accumet AR20 pH meter calibrated with pH 4 and pH 7 buffers after every 20 samples and with every tenth sample replicated. pH was measured for both topsoil samples and subsoil samples.

4.2.2. Total Soil Carbon and Nitrogen

Soil samples were further ground to a very fine powder using a planetary ball mill. 0.2 g of sample were weighed out into a porcelain boat. 1 g of Comcat accelerator was added and mixed thoroughly with the sample. Carbon and nitrogen content of the soils were then determined using a LECO CNS-2000 elemental analyser.

4.2.3. Soil Anions and Cations

For each site, bulked air-dried, ground soil samples were made up using 2 g of topsoil from each quadrat to give a total of 10 g. The soil was placed in a leaching tube and leached with 100 ml 1M KCl. KCl standards were also included. The extracted soil solution was analysed for ionic content using a Dionex DX100 Ion Chromatograph (IC) with IonPac CS16 column for cations and IonPac AS9-HC column for anions. Peak areas were measured and concentrations in mg l^{-1} were calculated using a calibration curve.

4.2.4. Plant Available Phosphorus

Bulked samples for each site were made up using approximately 1 g of topsoil from each quadrat to give a total of 5 g. A standard Olsen extraction was used with an ascorbic-ammonium molybdate colour complex as described by MAFF (1986) taking on board the comments of Gilbert (2001). Absorbance was determined using a Heios Thermo Spectropic colorimeter at a wavelength of 880nm. A calibration curve was used to calculate concentrations in mg g^{-1} .

4.2.5. Metals

The KCl extract used for soil anions and cations was also used for heavy metal determination. The extracted solution was diluted to 1 in 50 with deionised water and acidified using 0.2 ml of concentrated nitric acid. Metal concentrations were determined using an Agilent 7500a Inductively Coupled Phase Mass Spectrometer (ICP-MS).

4.2.6. Bulk Density

Bulk density of the soils was determined using 10 cm deep, 5 cm diameter soil cores. The soil was extracted from the aluminium cores and the middle 5 cm retained to calculate bulk density. The remainder was discarded as the top and bottom of the cores often had some soil missing. Cores were weighed and dried in an oven overnight at 103°C (Blake and Hartage, 1986). The cores were weighed again and the following formula used to calculate wet and dry bulk density:

$$\text{Bulk Density} = \text{Mass/core volume} \quad (\text{Eq. 4.1})$$

4.2.7. Potential Net Mineralization

Fieldwork for mineralization was carried out during July 2003. Samples were collected from a randomly selected sub-sample of the sites already visited and from the eight new sites. Samples were taken at randomly selected locations within the 1 ha sampling area. The turf was lifted using a spade and paired 5cm cores with a diameter of 5 cm were hammered in with a dolly and cutter. The cores were trimmed and sealed in a plastic bag, then stored in iceboxes until they could be returned to the laboratory.

One of the cores from each pair was extracted immediately and the second was used for incubation. The cores for incubation were soaked in a tray of water until saturated. Once saturated, they were placed on a tension plate with a constant level device overnight to attain –50cm tension. The cores were covered with Parafilm to prevent drying during incubation and then weighed. Cores were incubated at 35°C for 14 days. On completion of the incubation cores were weighed again to assess the loss of moisture during incubation.

For extraction, soil was removed from the core and homogenised, with large stones being removed by hand. 25 g of soil were removed and extracted with 50 ml of 1M KCl. Full methods are given in Stanford and Epstein (1974). The filtrate was stored in centrifuge tubes and frozen. The remainder of the soil was oven dried at 103 °C and weighed to determine soil dry weight.

For reasons that are unclear (perhaps due to sample storage) no nitrate could be detected in the samples. Therefore it was not possible to measure nitrate mineralization. The following calculation was used to calculate ammonium mineralization:

Calculation of soil moisture content:

$$\text{Soil moisture content (ml l}^{-1}\text{) (25g soil)} = \frac{\text{soil dry weight} - \text{soil wet weight}}{\text{soil wet weight}} \times 25 \quad (\text{Eq. 4.2})$$

Total volume of extractant solution used:

$$\text{Volume of extractant (ml)} = 50 - \text{soil moisture content} \quad (\text{Eq 4.3})$$

Amount of NH_4^+ mineralized per volume of soil extracted:

$$\text{Mineralized } \text{NH}_4^+ \text{ (mg kg}^{-1}\text{)} = \frac{\text{NH}_4^+ \text{ (mg l}^{-1}\text{)} \times (\text{volume of extractant})}{(25 \times 1 - \text{proportion of water})} \quad (\text{Eq.4.4})$$

Conversion from gravimetric (mg kg⁻¹) to volumetric (kg m⁻³) using bulk density of soil:

$$\text{Mineralized } \text{NH}_4^+ \text{ (kg m}^{-3}\text{)} = \frac{\text{Mineralized } \text{NH}_4^+ \text{ (mg kg}^{-1}\text{)} \times \text{Bulk density (kg/m}^3\text{)}}{10^6} \quad (\text{Eq 4.5})$$

Cores which had lost substantial amounts of moisture (over 40%) were discounted at this stage.

4.2.8. Nitrogen isotopes

Total nitrogen and $^{15}\text{N}/^{14}\text{N}$ ratios in the soil samples were determined by dry combustion on a Carlo Erba CN 2500 analyser coupled with Thermoquest Delta^{plus} continuous-flow isotope ratio mass spectrometer. Natural abundance of ^{15}N was expressed as $\delta^{15}\text{N}$ [‰]_{AIR}, which represents the ratio of $^{15}\text{N}:^{14}\text{N}$ relative to the international standard. The analytical precision of the measurements was 0.17 ‰.

4.2.9. Data analysis

Multiple regressions between soil chemical constituents and potential environmental drivers were carried out as described in section 3.2.2. The independent predictor variables used in the regressions on soil chemistry were: five meteorological variables (MAP, MAT, AE, PE, SMD), nine deposition chemistry variables (N dep, NH_x dep, NO_x dep, wet dep NO_3 , wet dep NH_4^+ , dry dep NO_2^- , dry dep NH_3 , acid dep and S dep) and six environmental variables (altitude, litter, slope, aspect, grazing and enclosure) (see table 3.1).

4.3 Results

4.3.1. Soil pH

Topsoil pH decreases with increasing N deposition ($r^2=0.33$, $p<0.01$) (equation 4.6, figure 4.1). Breaking this relationship down further reveals that total NH_x deposition is the most important component of N deposition for topsoil pH, explaining approximately 30% of the variation. S deposition added an almost equal amount of variation as N deposition (equation 4.7, figure 4.2). No other variables were significant.

$$\text{Topsoil pH} = 4.92 - 0.027(\text{N dep}) \quad (r^2 = 0.33, p<0.01) \quad (\text{Eq. 4.6})$$

$$\text{Topsoil pH} = 5.11 - 0.021(\text{Ndep}) - 0.019(\text{Sdep}) \quad (r^2 = 0.65, p<0.001) \quad (\text{Eq. 4.7})$$

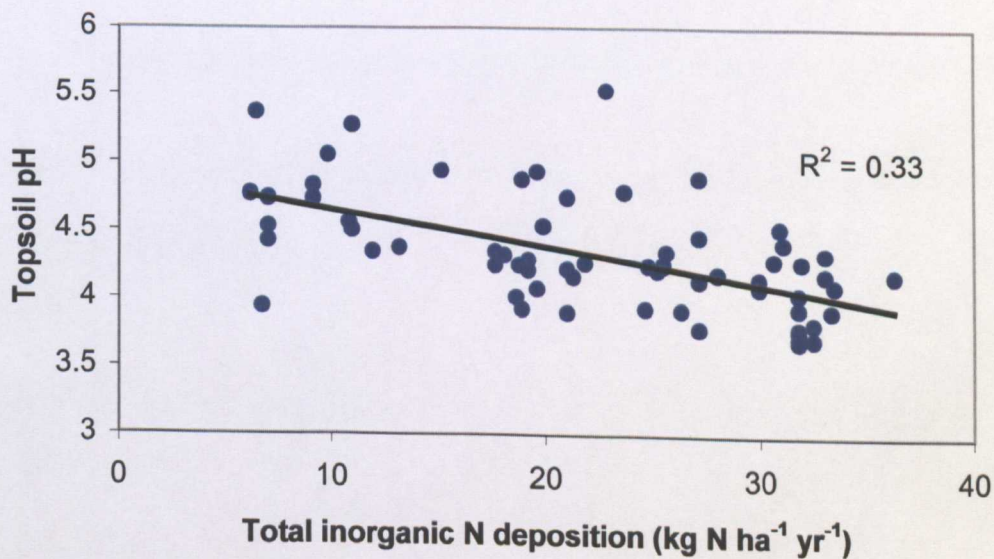


Figure 4.1 Decline in topsoil pH with increasing N deposition.

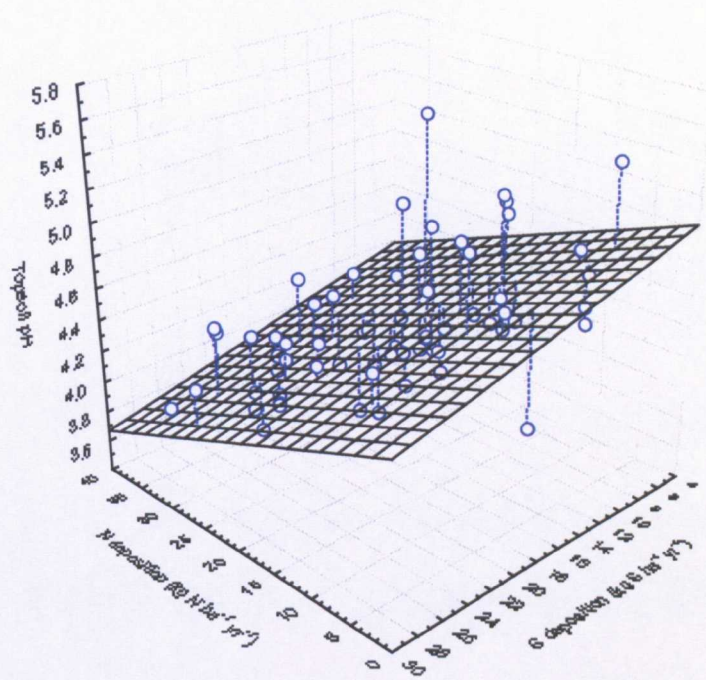


Figure 4.2 Decline in topsoil pH with increasing N and S deposition.

Subsoil pH is also most closely correlated with N deposition ($r^2=0.33$, $p<0.01$) (equation 4.8, figure 4.3).

$$\text{Subsoil pH} = 5.13 - 0.021(N_{\text{dep}}) \quad (r^2 = 0.33, p<0.01) \quad (\text{Eq. 4.8})$$

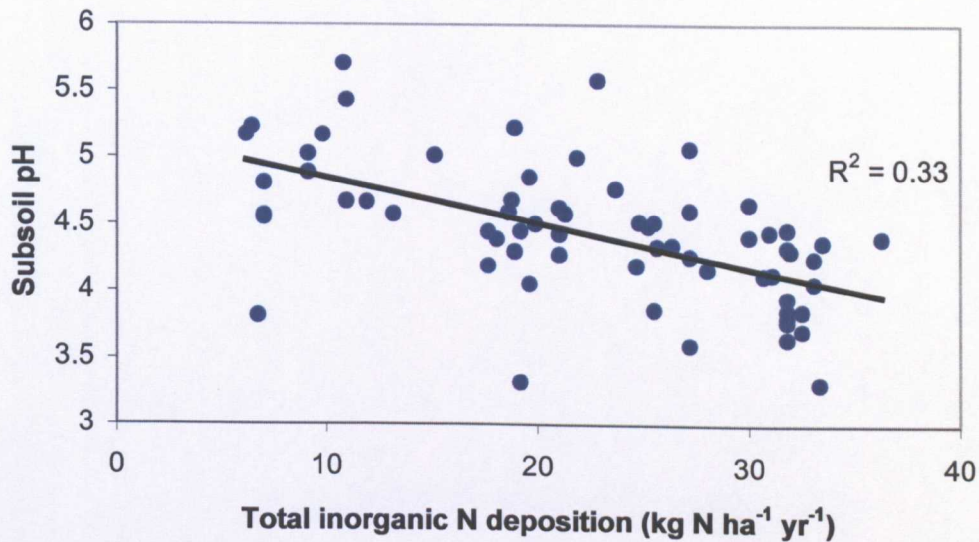


Figure 4.3 Decline in subsoil pH with increasing N deposition.

A breakdown of the total N deposition reveals that total NH_x deposition is the most important variable, explaining 29% of the variability in subsoil pH, followed by dry deposition of NO_2 accounting for a further 5%.

4.3.2. Total Soil Carbon and Nitrogen

There was no significant relationship between topsoil N (%) and total inorganic N deposition ($p = 0.53$) (figure 4.4). There is a weak negative relationship between the topsoil C:N and total N deposition ($r^2 = 0.08$, $p < 0.05$) (figure 4.5). Stepwise regression with climate variables shows that N deposition is the most important variable with the effects of climate removed.

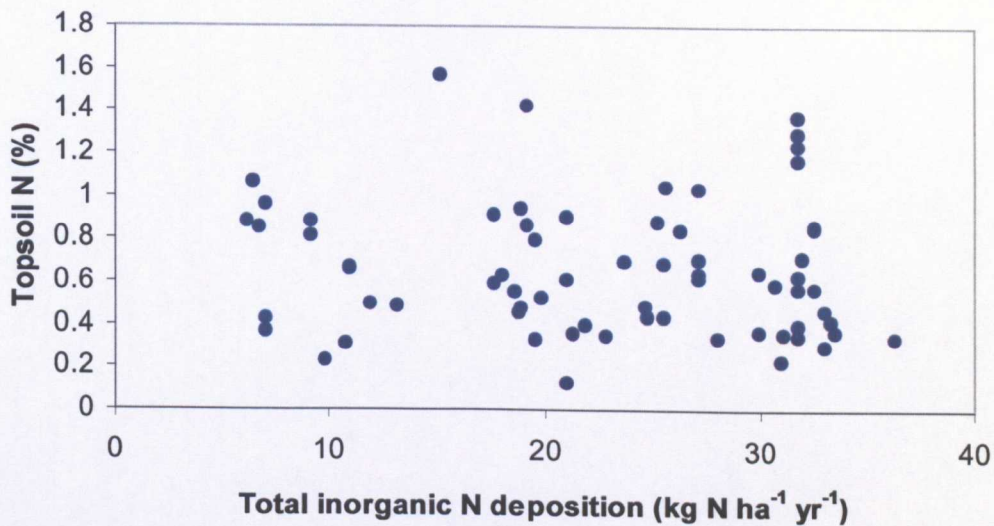


Figure 4.4 Topsoil N in relation to total inorganic N deposition.

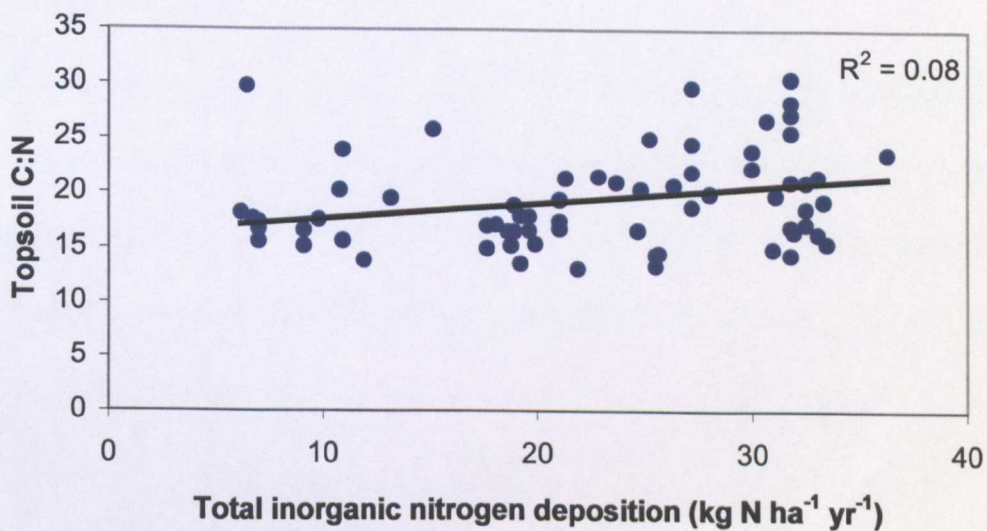


Figure 4.5 Topsoil C:N in relation to total inorganic N deposition.

4.3.3. Nitrate and Ammonium

Soil nitrate did not show any significant linear relationship with total NO_x deposition or wet deposition of nitrate (figure 4.6). There was a significant linear relationship with total inorganic N deposition ($r^2 = 0.12$, $p < 0.01$). However, if 3 outlying points were removed, the relationship was no longer significant.

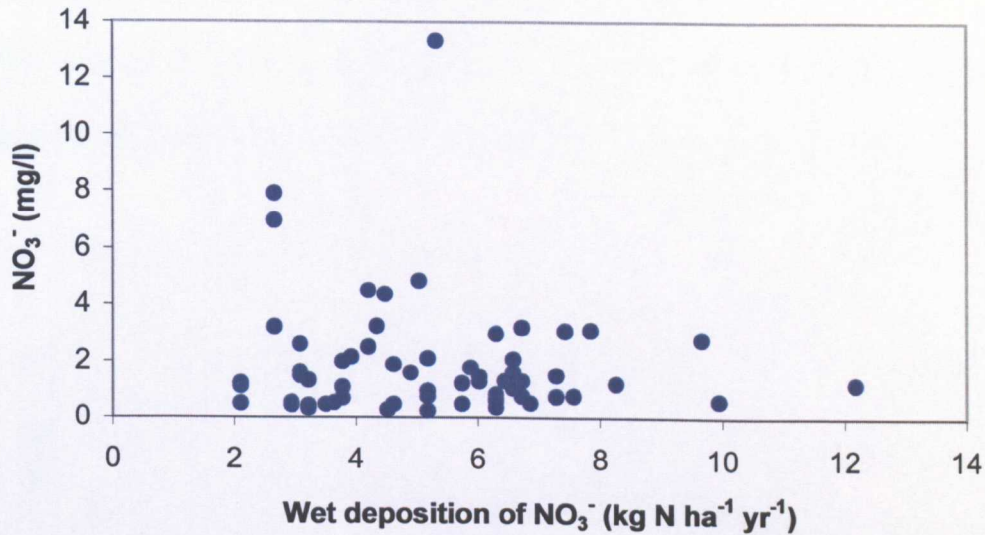


Figure 4.6 KCl extractable nitrate in relation to wet deposition of NO_3^- .

Soil ammonium shows a much better linear relationship with nitrogen deposition. Soil ammonium is significantly related to wet deposition of NH_4^+ ($r^2 = 0.36$, $p < 0.01$) (figure 4.7a) and total NH_x deposition ($r^2 = 0.34$, $p < 0.01$). There is also a significant relationship with total inorganic N deposition ($r^2 = 0.39$, $p < 0.01$) (figure 4.7b).

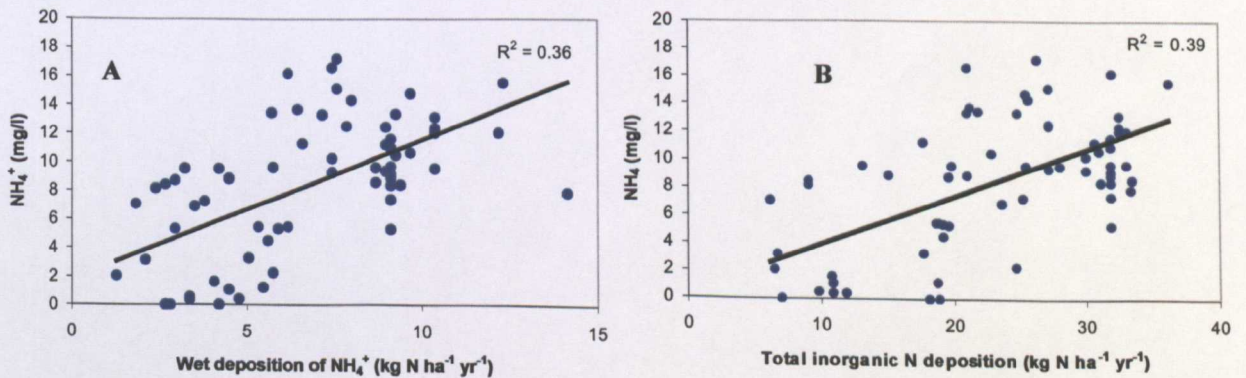


Figure 4.7 KCl extractable ammonium in relation to **A.** wet deposition of ammonium and **B.** total inorganic N deposition.

4.3.4. Plant Available Phosphorus

There was no significant relationship found between plant available soil phosphorus and total inorganic N deposition ($p = 0.15$) (figure 4.8).

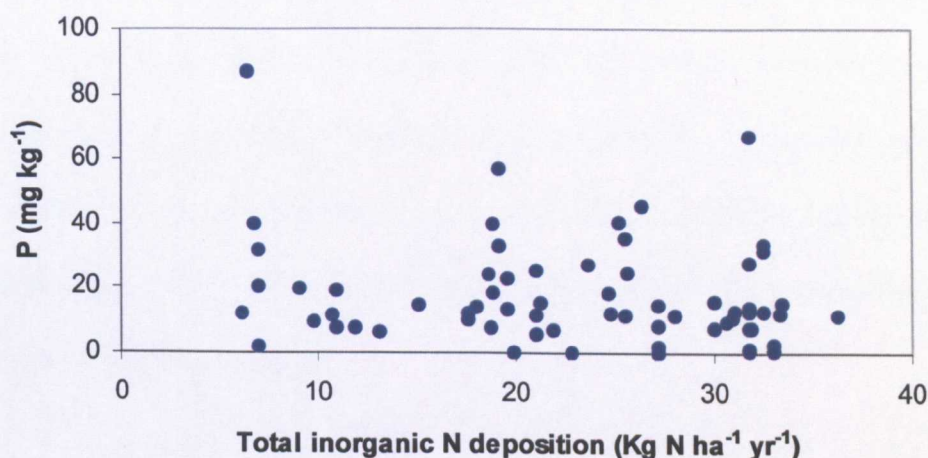


Figure 4.8 Topsoil P in relation to total inorganic N deposition .

4.3.5. Metals

The concentrations of the following extractable metals were measured in the topsoil and subsoil and the following significant linear relationships found (tested by regression analysis):

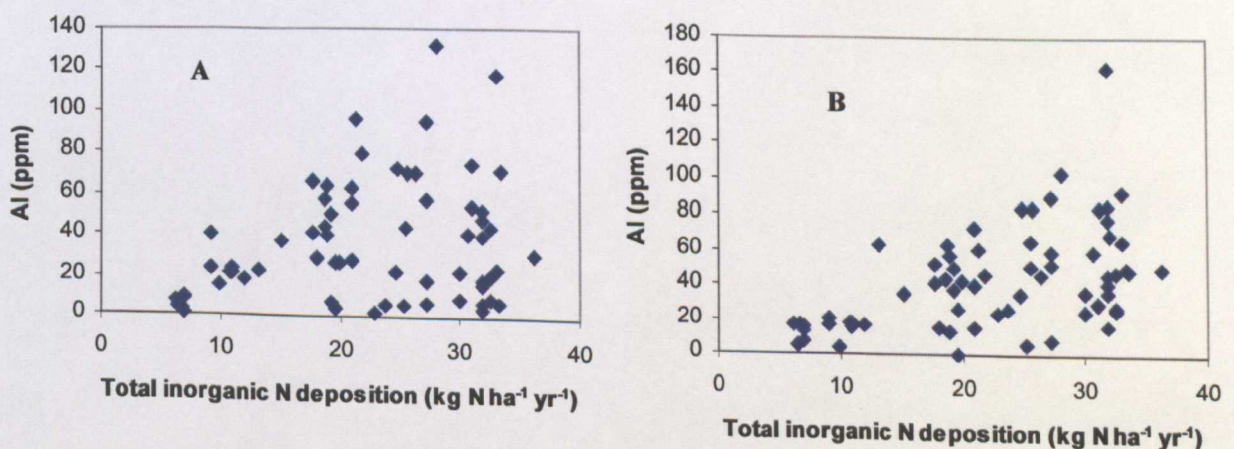
Metal	Topsoil		Subsoil	
	Total N deposition	Topsoil pH	Total N deposition	Subsoil pH
Lithium	** (-)	** (+)	** (-)	** (+)
Sodium	** (-)	** (+)	** (-)	* (+)
Magnesium	** (-)	** (+)	* (-)	
Aluminium	* (+)		** (+)	** (-)
Calcium				* (+)
Chromium	** (-)			
Manganese		* (+)		
Iron				
Nickel	** (-)			
Copper				
Arsenic	** (+)	** (-)	** (+)	** (-)
Barium	* (-)			** (+)
Lead	** (+)	** (-)	** (+)	** (-)

Table 4.1 Relationships between metals and total inorganic N deposition and soil pH
(* $p < 0.05$, ** $p < 0.01$; -/+ indicates the direction of change).

Lithium showed a significant negative trend with N deposition and a positive trend with pH in both the topsoil and the subsoil, reflecting higher concentrations of extractable lithium at low N deposition or higher pH. Sodium and magnesium show a similar trend. Subsoil calcium showed a significant positive relationships with pH, but; no other significant correlations with Ca^{2+} were demonstrated. Manganese concentration is positively correlated to topsoil pH only. Chromium and nickel show an increase at lower N deposition, but, this trend is mainly influenced by several outliers. An outlier similarly exercises the main control in a significant increase for barium at higher subsoil pH.

Aluminium showed the reverse trend, with higher concentrations at lower pH. This trend was stronger in the subsoil than the topsoil, where the relationship is not significant. Aluminium was positively correlated with N deposition. Arsenic also (weakly) followed this pattern, as does lead, with clear wedge-shaped distribution (figures 4.9 and 4.10).

Iron and copper show no significant trends in the topsoil and subsoil with either N deposition or soil pH.



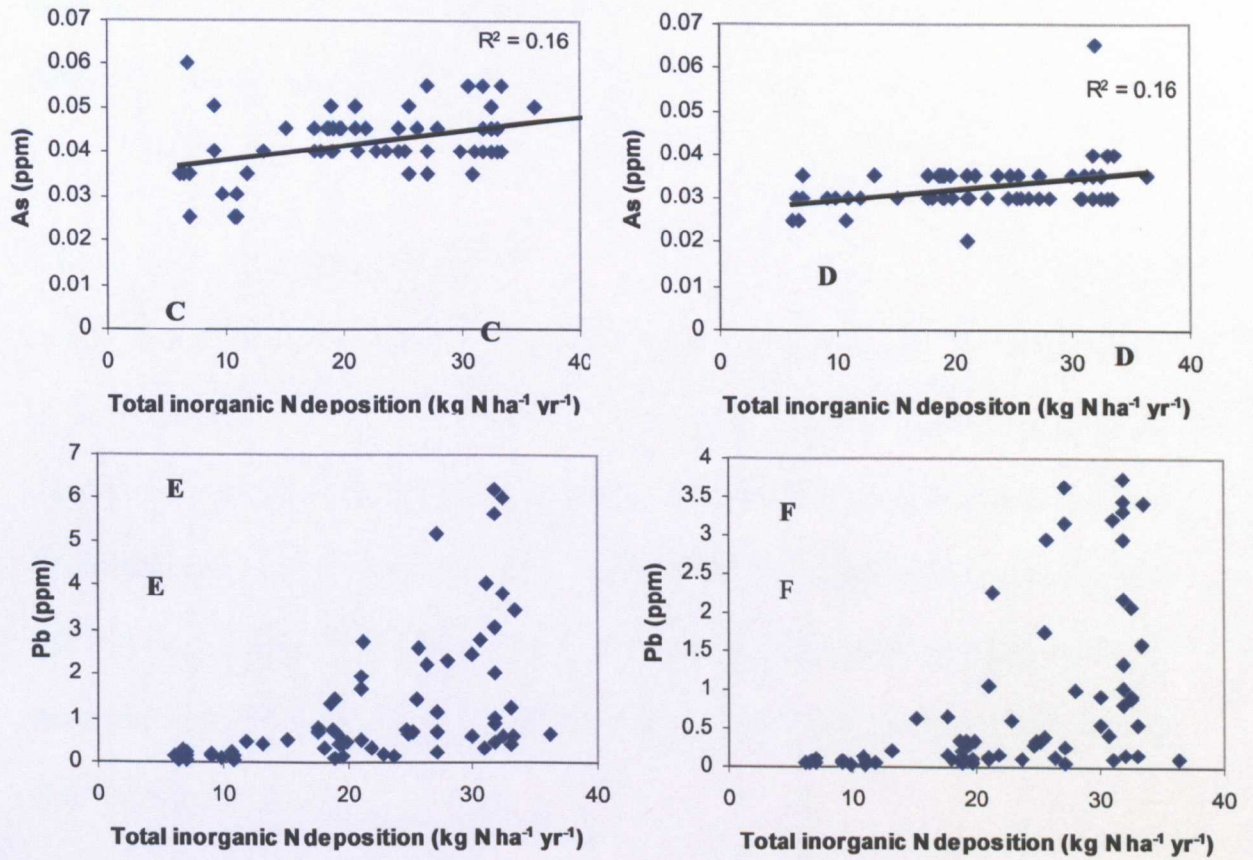


Figure 4.9 Aluminium concentration in (A) topsoil and (B) subsoil; Arsenic concentration in (C) topsoil and (D) subsoil; and Lead concentration (E) topsoil and (F) subsoil against N deposition.

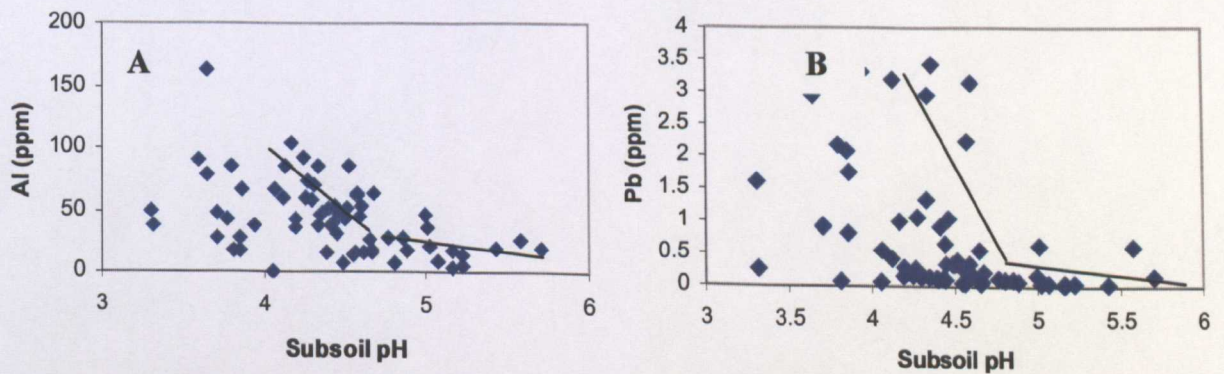


Figure 4.10 Aluminium (A) and Lead (B) concentration in subsoil against subsoil pH.

There is a weak but significant relationship between topsoil $\text{Al}^{n+}:\text{Ca}^{2+}$ ratio and N deposition ($r^2 = 0.03$, $p < 0.04$) but not with topsoil pH ($p = 0.09$). For subsoil there are

weak but significant relationships between $\text{Al}^{n+}:\text{Ca}^{2+}$ ratio and both N deposition ($r^2 = 0.06$, $p < 0.03$) and subsoil pH ($r^2 = 0.06$, $p < 0.04$).

4.2.7. Potential Net Mineralization

NH_4^+ mineralization rate was very variable at some sites (figure 4.11). There was a slight trend between NH_4^+ mineralization and total inorganic N deposition although this was not significant (figure 4.12). Forward stepwise multiple regression shows that of the environmental and soil variables measured (table 3.1 plus soil pH, soil C, N, P and NH_4^+) potential evapotranspiration accounts for the most variation in NH_4^+ mineralization (22%) followed by subsoil pH. In total they explained 50% of the variation (equation 4.9).

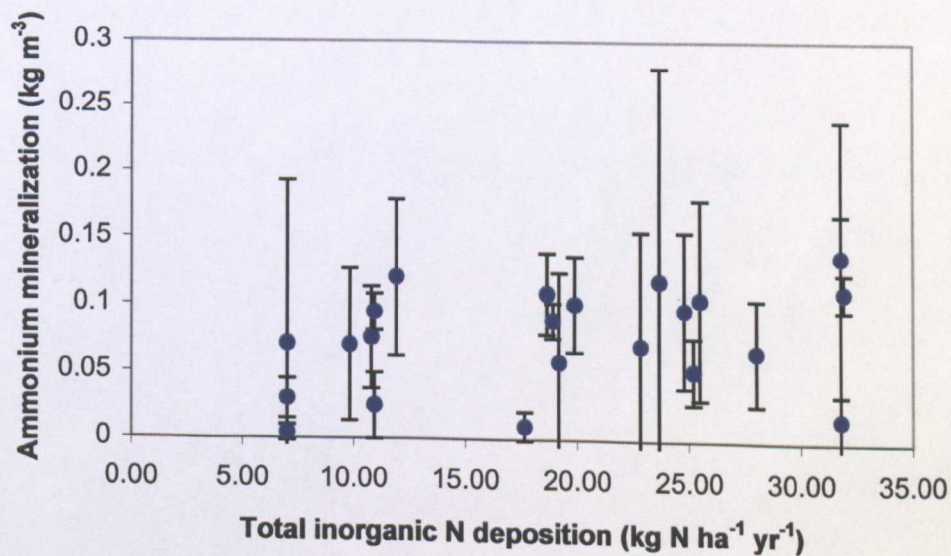


Figure 4.11 Mean ammonium mineralization against total inorganic N deposition. Error bar shows standard deviation.

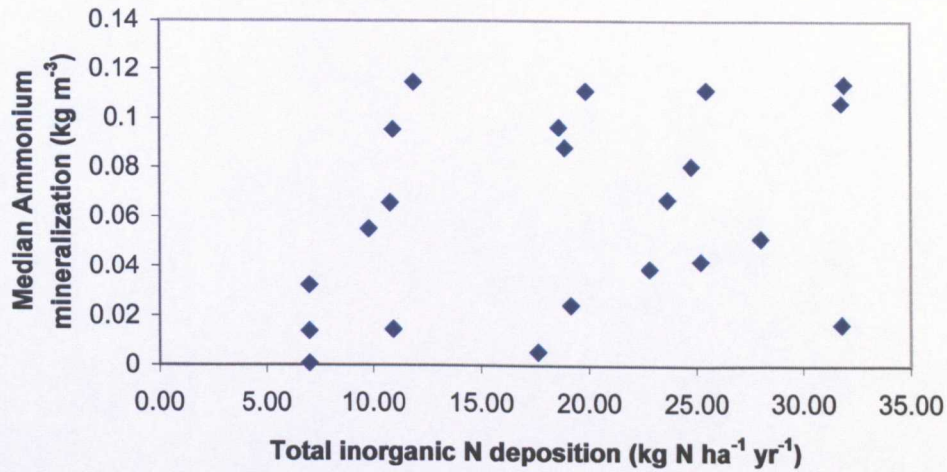


Figure 4.12 Median ammonium mineralization against total inorganic N deposition.

$$\text{NH}_4^+ \text{ Mineralization} = 0.57 - 0.0059 (PE) - 0.052 (Sub \text{ pH}) \quad (r^2 = 0.51, p < 0.01)$$

(Eq. 4.9)

4.2.8. Nitrogen isotopes

There is a slight linear positive relationship between natural abundance of soil $\delta^{15}\text{N}$ and total inorganic N deposition ($r^2=0.07$, $p<0.05$) although a log curve shows a better relationship (figure 4.13). However, if the largest outlier is removed there is no longer any significant trend ($p = 0.40$). There also appears to be a slight positive correlation between $\delta^{15}\text{N}$ and topsoil C:N (figure 4.14) however this is also not significant ($p = 0.09$).

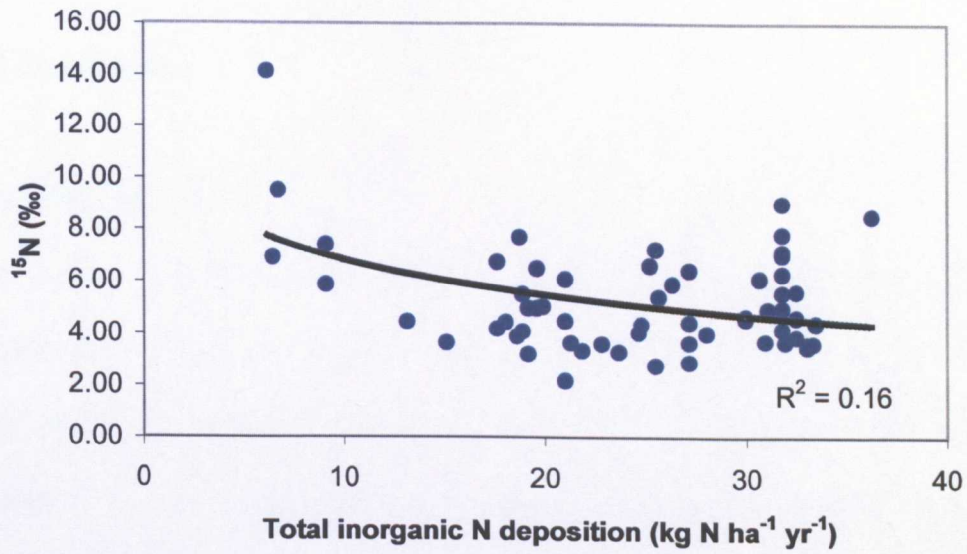


Figure 4.13 Natural abundance of $\delta^{15}\text{N}$ in relation to total inorganic N deposition.

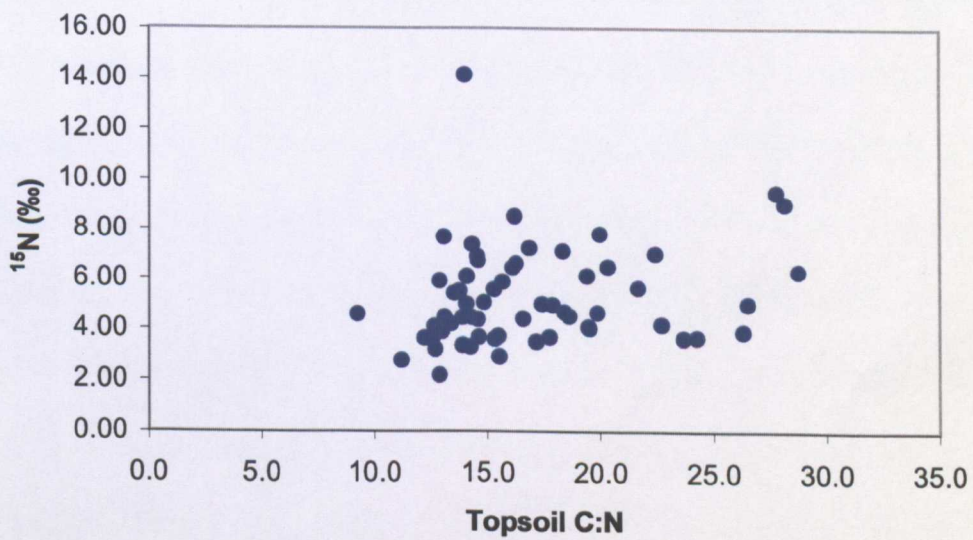


Figure 4.14 Natural abundance of $\delta^{15}\text{N}$ in relation to C:N.

4.4 Discussion

4.4.1. Soil pH

The decline in topsoil and subsoil pH along the gradient of N and S deposition (figures 4.1 - 4.3) is an effect we would expect to see. N deposition in the form of NH_4^+ has the potential to acidify soils via nitrification, uptake and ion exchange (see section 4.1). SO_2 and NO_x have the potential to acidify through oxidation to H_2SO_4 and HNO_3 . Both NO_3^- and SO_4^- can leach basic cations from the soil, resulting in chronic acidification (Johnson *et al.*, 1986). A number of studies have shown declines in soil pH over time periods ranging from 17-100 years in British and European woodlands and grasslands (Farmer, 1995; Kuylensstierna and Chadwick, 1991; Falkengren-Grerup *et al.*, 1987 and Blake *et al.*, 1999) and along gradients of acid deposition (Lundstrom *et al.*, 1998 and Skiba *et al.*, 1989). Skiba *et al.* (1989) found a significant correlation between the acidity of peats in Scotland and acid deposition. This study confirms the importance of acid deposition to soil pH. A further breakdown of the results suggests that N deposition is currently as important as S deposition in causing soil acidification in the UK (figures 4.7 and 4.8). During the last two decades the relative contribution of N to acid deposition has increased considerably such that in 1997 it accounted for over 65% of acid deposition (NEGTA, 2001). Of the components of nitrogen deposition, total NH_x deposition is the most important variable, and in the majority of the sites reduced N was very much higher than oxidised N (figure 4.15).

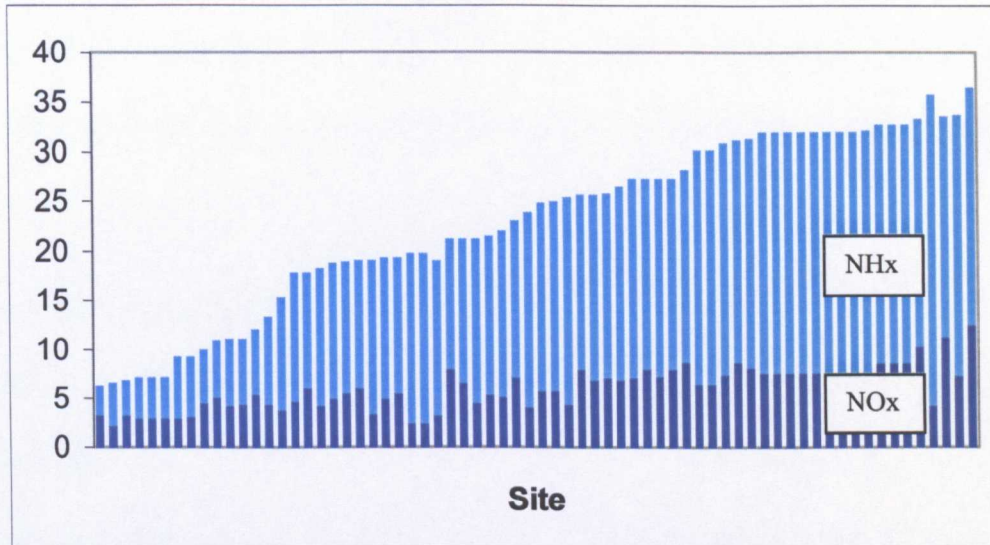


Figure 4.15 The relative importance of NO_x and NH_x deposition.

Of all the possible environmental drivers considered, subsoil pH was most closely correlated with N deposition (figure 4.3). N deposition explains 33% of the variation, with S deposition explaining only a further 5%. The difference in the relative importance of sulphur deposition to pH in the topsoil and subsoil may be due to adsorption of sulphate by the iron and aluminium oxides abundant in the B-horizon of podzols (Chao *et al.*, 1962). In the present research subsoil samples were taken at a depth of 30-40 cm, which is most likely to be below this level of adsorption in the lower B-horizon. Podzols are common in the uplands of Britain and commonly support U4 grasslands (Rodwell, 1992).

As with topsoil, total deposition of ammonia is the most important of the N deposition variables in influencing pH, followed by NO_2 (equation 4.7). NO_2 is oxidised within or on the surfaces of vegetation or soil, generating acidity. Dry deposition of NO_2 forms a comparatively small fraction of the total N deposition, and it is therefore

interesting that it should appear important, although dry deposition is concentrated around industrialised areas. The importance of dry deposition of NO_2 to subsoil pH may be caused by flushes of acidity being washed into the subsoil during heavy rains when it has been collecting on the surface as a dry deposit for a period of time.

4.4.2 Total Soil Carbon and Nitrogen

There is no significant relationship between percentage N in the topsoil and total N deposition. One might expect an increase in soil nitrogen with increasing N deposition, but the situation for these soils is more complex than this. Total N in the soil includes both organic and inorganic forms of N and is affected by many processes in the soil, including the rate of organic matter breakdown and leaching. The soils analysed varied considerably in their type and structure ranging from very sandy soils through to peats. These soils have very variable organic matter contents, which would be expected to cause major differences in the nitrogen content, since organic matter is where the highest concentration of soil N is generally found.

The findings are similar to those of Falkengren-Grerup *et al.* (1998) who found no consistent changes in the soil total N concentration in woodlands (5 cm depth) at different levels of N deposition. Experimental additions of N have also failed to show any relationship between soil N and N application. Morecroft *et al.* (1994) found total N did not differ significantly even with treatments up to $140 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ after 1 year. They suggested increased plant uptake, leaching or denitrification as potential reasons for this, although later work on the same sites has ruled out leaching as a potential cause (Phoenix *et al.*, 2003). Marcos *et al.* (2003) also report no change in soil total N with N applications of $56 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ after 3 years. The apparent lack

of response in experimental systems may be because applications were only made over a short time.

C:N ratio does show some correlation with N deposition although this is very weak ($r^2=0.08$, $p<0.05$). Climate is a very important influence over soil C:N ratio. Running a multiple regression including either soil moisture deficit (incorporates both temperature and rainfall) or both temperature and rainfall as separate factors does not increase the r^2 of the model and N deposition is still significant. C:N ratio has been demonstrated as an important factor in NO_3^- leaching in forests (MacDonald *et al.*, 2002, Gundersen *et al.*, 1998, Dise *et al.*, 1998) and has been shown to have an influence over the incidence of nitrophilic plants (Hofmeister, 2002).

In North American forests a significant relationship between N deposition and C:N ratio has been identified along a gradient of N deposition, especially in the organic horizon, with a weaker trend in mineral horizon. In that study Aber *et al.* (2003) stated that a large degree of variability in the C:N ratio is influenced by other factors, although only the effects of temperature were removed.

Falkengren-Grerup *et al.* (1998) examined C:N ratios within soil pH classes to look for interactions with N deposition between two regions of Sweden with differing N deposition. Using analysis of variance followed by Tukey's non-additivity test they found no consistently significant differences in C:N ratio between pH classes of 3.1-3.5, 3.5-4.0, 4.0-4.5, 4.5-5.0 and 5.0-7.7. Tukey's non-additivity test provides a pairwise comparison of means of the C:N ratio by pH class. Using 4 of these classes (3.5-4.0, 4.0-4.5, 4.5-5.0 and 5.0-7.7; none of the soils in this dataset have pH lower

than 3.5) and the same statistical tests showed that in the UK the C:N is significantly higher ($F = 3.73$, $p < 0.05$) in the lowest pH class as compared to the other pH classes.

4.4.3. Nitrate and Ammonium

The lack of a significant relationship between soil nitrate and deposition of NO_x , NO_3^- (figure 4.6) or total inorganic N deposition inputs is likely to be due to a number of reasons including the efficiency of plant uptake and denitrification in wetter soils. It may also be due in part to the high mobility of nitrate in the soil and so may be being leached to lower soil horizons, as demonstrated with the importance of nitrate for subsoil pH. We may be seeing leaching through the profile with microbial immobilisation preventing the nitrate being lost from the system entirely. At higher N inputs nitrate may be leaching more rapidly to lower soil horizons rather than accumulating in the topsoil. Nitrate leaching has been shown to be related to N inputs in forest soils (Dise and Wright, 1995). Although N addition experiments in forests (Emmett *et al.*, 1998) and grasslands (Phoenix *et al.*, 2003) show very low rates of leaching, this may be due to the period of N application being insufficiently long to bring about a response. An alternative explanation is that the variability in the soils, for example in organic matter content, is responsible for the observed differences.

At three sites high soil nitrate is associated with low nitrate inputs (figure 4.6), which may be due to a conversion of NH_4^+ to NO_3^- due to high nitrification and mineralization rates since they are not sites with high total N deposition. Two of the three sites have high grazing intensity and it is possible that the observed pattern results from high N turnover due to grazing animals. The third site is adjacent to a

picnic area; the activities of visitors to the site may be contributing to elevated nitrate in the soil.

The positive correlation between ammonium concentration and ammonium deposition (figure 4.7) may reflect the greater storage of NH_4^+ in the soils. The strongest correlation is with total N deposition, perhaps reflecting an interaction with soil microbial processes and a reduction process. These results, showing a retention of NH_4^+ in the topsoil but not of NO_3^- are consistent with a number of N addition experiment to forested ecosystems (Buchmann *et al.*, 1996; Emmett *et al.*, 1995; Magill *et al.*, 1997 and Stuanes and Kjonaas, 1998). Application of ^{15}N labelled ammonium and nitrate demonstrated a higher immobilisation of ammonium and adsorption onto exchange sites of organic matter and clay. Conversely nitrate was lost to groundwater and 8 months after the application of ^{15}N tracer to a forested catchment could be detected in greater proportions in the subsoil than the topsoil (33% v 25% of the input) (Buchmann *et al.*, 1996). These results, combined with those of the previous section, provide good support that observations made in experimental N manipulations are actually occurring in UK acid grasslands.

4.4.4. Plant Available Phosphorus

The lack of relationship between plant available phosphorus and total inorganic N deposition is an indicator that there is no clear change in the soils. Considering the variation in the soil types (discussed in section 4.4.2) this is of little surprise. Turner *et al.* (2003) suggest a change in the form of P in the soil with changes in soil pH and N deposition leading to an increase in recalcitrant soil organic P. This may affect the

availability of P to some species of plant and hence their competitive ability and should be an area for future work.

4.4.5. Metals

Those metals which show a positive correlation with pH and a negative correlation with total N deposition fall into two groups. The base cations occur in relatively large amounts in the soil and include sodium, magnesium and calcium. They are commonly found at higher levels in higher pH soils (Ashman and Puri, 2002), and can leach from the soil at low pH (Bergholm *et al.*, 2003). A number of studies have demonstrated a decline in base cations with increasing soil acidity related to N deposition and N addition (e.g. Jonsson *et al.*, 2003; Nohrstedt, 2002). These elements are also able to exert an influence over the acidity of the soil by interaction with the cation exchange complex. Calcium does not show strong relationships with pH or N deposition in the present study, which may be due the low quantities found in these acid soils.

Other metals that show a positive correlation with N deposition (lithium, chromium, manganese, nickel and barium) are only found at trace levels in the soil insufficient to exert influence over the cation exchange complex. There may be a mobilisation effect where these metals are dissociated from the cation exchange complex at higher pH (Tyler and Olsson, 2001). However, with the exception of lithium, for these metals the relationships were neither strong nor consistent and in the case of chromium, nickel and barium were controlled mainly by a few high values at low deposition sites (within range of naturally occurring concentrations; Kabata-Pendias and Pendias, 1992). Consequently chromium, manganese, nickel and barium could be grouped

together with iron and copper where there is no significant effect of pH or N deposition.

Aluminium, arsenic and lead show the opposite trend, and these metals are probably being mobilised as the soil becomes increasingly acidic. The mobilisation of aluminium and lead is consistent with experimental acidification of soils (figure 4.10) (Tyler and Olsson, 2001, Kanata-Pendias and Pendias, 1992) and the increased mobilisation of aluminium with N deposition and fertilisation is well recorded (e.g. Blake *et al.*, 1999; Dise *et al.*, 2001; Emmett *et al.*, 1995; Mulder and Stein, 1994). As the pH of soils falls below 5.0, mobilisation of aluminium (Al) in several forms begins from the silicate lattices associated with clay minerals and organic materials. Below a pH of about 4.5, in the Al buffer range, interlayer Al is weathered, releasing Al^{3+} (Ulrich, 1991). This effect is apparent in figure 4.10. As pH continues to decline, a greater proportion of Al^{n+} is as the most toxic species, monomeric Al^{3+} . Soil pH is buffered at around pH 4.2 by the release of Al^{3+} . Lead is also mobilised at low pH, as can be clearly seen in this dataset (figure 4.10). The influence of a factor in addition to acidity is demonstrated in the wedge-shaped distribution of lead versus N deposition. However, multiple regression shows that no other measured environmental variables explain additional variation in topsoil or subsoil lead content.

A weak correlation between metal concentration and pH but not N deposition occurs in several instances. In these situations (manganese in the topsoil and calcium in the subsoil), the changes may be due to pH effects that are not as a result of N deposition. Many factors influence soil pH including other acid deposits, microbial processes, soil parent material, management and plant-soil interactions. Although there is a

correlation between N deposition and pH (section 4.4.1), there is a considerable amount of variation in pH that is not explained by N deposition. Consequently it is not surprising that there are occasions where metal concentration correlates with pH and not N deposition. There are several metals where there is correlation with total N deposition but not pH (aluminium, barium, chromium and nickel in topsoil and magnesium in subsoil). The effects that we see are very likely to be due to pH but complicated by factors such as organic matter content of the soils.

Organic matter is also likely to be a factor where there are correlations between metal concentration and either pH or N deposition in the topsoil but not in the subsoil (chromium, manganese and nickel) or subsoil but not topsoil (calcium). Organic matter can bind metals in the soil, causing them to be more mobile in the subsoil (Asman and Puri, 2002).

4.4.6. Potential Net Mineralization

Nitrogen mineralization is the conversion of organic N in the soil organic matter to inorganic N. The result is an increase in nitrate and ammonium available to plants. Although there appeared to be a slight trend in the data for increasing NH_4^+ mineralization with increasing N deposition this was not significant (figure 4.12). The data showed a large amount of small-scale spatial variability in the mineralization rates (figure 4.11). This variability has been identified in a number of previous studies (Lovett and Rueth, 1999; Emmett *et al.*, 1995 and Bogaert *et al.*, 2000).

Despite such variability, a number of investigations have found a relationship between N mineralization and N deposition both in experimental N application studies (Magill

et al., 1997; Morecroft *et al.*, 1994 and Wedin and Tilman, 1996) and gradient studies (Diekman *et al.*, 1998; Diekmann and Falkengren-Grerup, 1998 and Falkengren-Grerup *et al.*, 1998) in both grasslands and forests. In this case, despite the measurement of potential NH_4^+ mineralization by laboratory incubation, which ensures comparability, the effects of the national climatic gradient are still evident.

In contrast to the minimal climatic variation in the Swedish studies (Diekman *et al.*, 1998; Diekmann and Falkengren-Grerup, 1998 and Falkengren-Grerup *et al.*, 1998) the climatic variation on the gradient of N deposition in the UK is considerable. This possibly explains the absence of a relationship between N deposition and mineralization in this study. The mineralization rate was much more closely related to potential evapotranspiration (negative correlation) and subsoil pH (negative correlation) which together explained 50% of the variation in mineralization. There is no direct explanation for this as potential net nitrogen mineralization was measured using laboratory incubations with standard conditions. There may be an indirect effect of soil type or carbon content, influenced by climatic variables.

Soil pH has been identified previously as an influence over NH_4 mineralization (Brunet *et al.*, 1998) and it is probably this relationship, rather than a direct relationship with N deposition, that is seen in the slight but non-significant trend with N deposition. Equation 4.9 shows that subsoil pH is related to N mineralization.

This study of N mineralization was possibly not carried out at the best time of year to obtain clear results. There are strong seasonal trends in N mineralization, even when varying incubation temperatures are controlled for (Davy and Taylor, 1974). Although mineralization rates are highest between June and August (these samples

were collected between late June and the end of July) they also tend to be more variable during this period (Carroll *et al.*, 2003). It has also been suggested that despite the difficulties associated with the variability introduced by field incubations they give much better results for N deposition. Laboratory incubations, can fail to show gradients that are present in the field (Gundersen *et al.*, 1998). Time and logistics did not permit field incubations, but this too should be an area of potential future study.

4.4.7. Nitrogen isotopes

Examining the $\delta^{15}\text{N}$ value can shed some light on the sources of nitrogen in the soil. Stable isotopes are measured as a ratio of the heavy and light isotopes in a sample, expressed using the delta (δ) notation. The relative amounts of $\delta^{15}\text{N}$ and ^{14}N vary predictably in soils and signature ratios can be used to identify the source of the nitrogen deposition (Nadelhoffer and Fry, 1994).

There is a weak but significant negative correlation between N deposition and the natural abundance of $\delta^{15}\text{N}$. Sites depleted in $\delta^{15}\text{N}$ tend to be those with high atmospheric N deposition (figure 4.13). This relationship is to be expected because atmospheric sources are relatively depleted in $\delta^{15}\text{N}$ (Nadelhoffer and Fry, 1994). If the outliers are removed there is no longer a significant trend, but without more data it is not possible to determine whether this trend is genuine. Emmett *et al.* (1998a) found no correlation between N deposition and $\delta^{15}\text{N}$ in European coniferous forests, suggesting other site characteristics such as land management and the amount of organic matter in the soil and consequences for the relative importance of immobilization relative to nitrification as possible cause for the lack of a trend.

Considering the variation in these factors between the sites in this investigation it is not surprising we do not see a significant trend.

4.5. Summary

In summary we can clearly detect the effects of acidification on the soils of U4 grasslands through the correlations between topsoil and subsoil pH and N deposition. This acidification has led to changes in the availability of metals. The effects of eutrophication through the amounts of N in the soil are less clear cut. There is a relationship between nitrogen inputs and NH_4^+ concentration, but this is not apparent for NO_3^- . There is also no relationship apparent between total soil N and N deposition, although the weak relationship with C:N ratio and the variability of the soils suggests that organic matter content may be partially responsible for this.

The lack of an effect identified with mineralization is most likely to reflect the variability of soil processes on a small scale rather than the presence or absence of an effect of N deposition.

5. Relationships between Nitrogen deposition and Plant tissue chemistry

5.1 Introduction

This chapter investigates the relationship between nitrogen deposition and plant tissue chemistry. Changes in the N content and N:P ratio of above-ground material in three species (a grass: *Agrostis capillaris*; a forb: *Galium saxatile* and a moss: *Rhytidiadelphus squarrosus*) are examined in terms of nitrogen inputs and environmental and soil variables. These species were selected for their high frequency and abundance in U4 grasslands (Rodwell, 1992).

Under the Global Atmospheric Nitrogen Enrichment (GANE) programme run by the Natural Environment Research Council tissue N content and N:P ratios were suggested as a key indicators of N deposition to be used by conservation agencies (GANE meeting, Edinburgh, 2003). The findings of this investigation will be discussed in light of this proposal. N:P ratios as indicators of nutrient limitation will also be used to examine the data collected.

5.2. Methods

Plant collection methods are described in section 2.3. Plant samples were dried at 55°C for 3 days on return to the laboratory. Samples were ground to <1mm.

5.2.1. Plant tissue Carbon and Nitrogen

For samples collected in 2002, 0.2g of sample was weighed out into a porcelain boat. 1g of Comcat accelerator was added and mixed thoroughly with the sample. Carbon and nitrogen content of the soils were then determined using a LECO CNS-2000 elemental analyser.

Due to breakdown of the LECO elemental analyser, samples collected during 2003 were analysed using an Elementar Vario EL111 elemental analyser. Approximately 0.3g of plant tissue was weighed out into a tin boat and rolled into a compact ball for analysis.

5.2.2. Plant tissue Phosphorus

A dry ashing method (Ryan *et al.*, 2001; Chapman and Pratt, 1961) was used for plant tissue P extraction followed by a standard Barton colour complex (MAFF, 1986). Absorbance was determined using a Heios Thermo Spectropic colorimeter at a wavelength of 410 nm. A calibration curve was used to calculate concentrations in mg g^{-1} .

5.2.3. Correction for seasonal variation

Due to the length of the sampling period (May to August) there was considerable variation in the growth stage of the plant tissues collected. To some extent this was compensated for by beginning the study with the most southerly sites and working north. Nonetheless it was clear from the growth stages of plants in the field that this was not sufficient to overcome the problem of seasonal variation.

Therefore, samples of each of the three species were collected approximately every two weeks between May and the beginning of September from a single location to provide information on the variation in the plant tissue nutrient content throughout the growing season. Samples were collected from U4 grassland at Stockgrove Country Park (grid reference SP919294) over a 1ha plot using the same methods as used in each of the field sites (section 2.3). The samples were analysed in exactly the same way as other samples. Three replicates were measured for each sample to provide a measurement replicate.

The levels of N and P in this plant material (figure 5.1) were then used to adjust the data collected from other sites according to day degrees. Scales based on time alone do not provide a measure of seasonal variation or ageing processes in plants because of the effects of temperature and the varying time of the onset of growth. Using day degrees gives a measure of physiological variation (O'Brien *et al.*, 1983; Allen *et al.*, 1979).

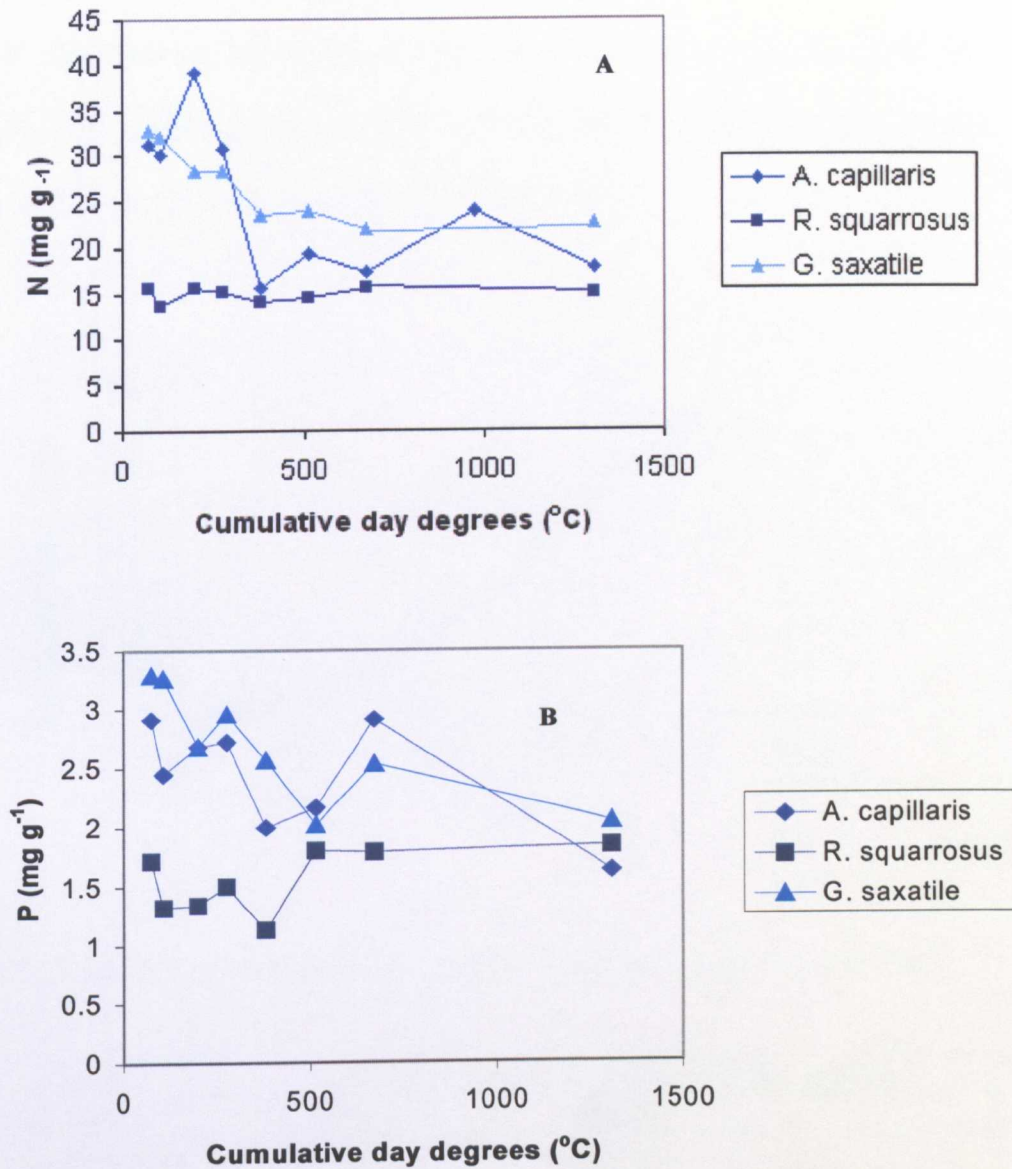


Figure 5.1 Levels of **A.** N and **B.** P (mg g⁻¹) in plant tissue samples collected from Stockgrove Park versus cumulative day degrees (°C) for *A. capillaris*, *R. squarrosus* and *G. saxatile*.

Cumulative day degrees were estimated for each site using the long-term (1971 – 2000) mean monthly temperatures (Meteorological Office, 2004). The mean monthly temperature was multiplied by the number of days in each month. When the daily

temperature exceeded 5°C, this was taken as the start of the growing period (Broad and Hough, 1993) and used for the beginning of the cumulative total for day degrees (figure 5.2). The cumulative day degrees were calculated for the dates each of the sites were visited (some examples are given in table 5.1).

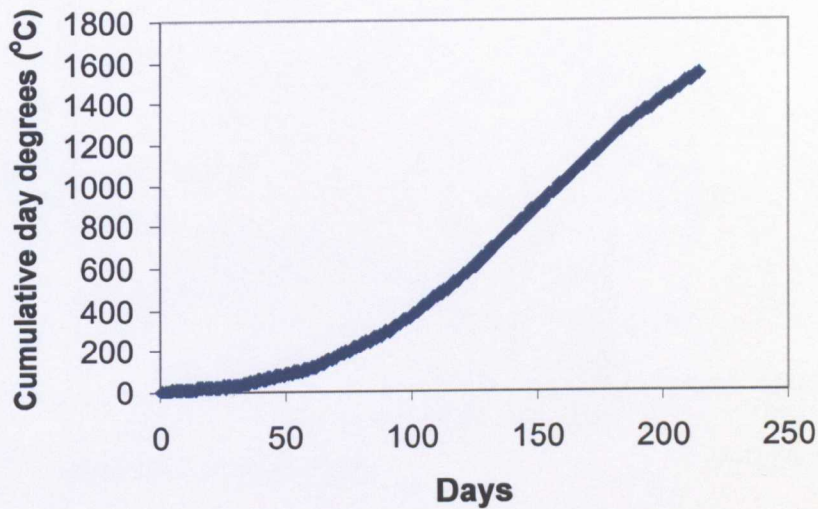


Figure 5.2 Example of cumulative day degrees calculated for Stockgrove Park.

Site	Location	Date of survey	Cumulative day degrees
42	Cornwall	27 May	346.25
30	East Midlands	23 June	516.5
1	North Scotland	21 August	663.45

Table 5.1 Example of cumulative day degrees calculated for 3 sites.

Day degrees for each of the visits to Stockgrove Country Park were calculated and plotted against tissue N and P. Equations for linear, log and exponential trends were tested and the one that gave the best fit was selected. As N and P content of tissues usually vary together (Broadley *et al.*, 2004), the same type of line was used for each species for both N and P.

A straight line gave the best fit for *Agrostis* ($N - r^2=0.38$, $p<0.01$; $P - r^2=0.44$, $p=0.07$). A log curve was selected for *Galium* ($N - r^2=0.80$; $P - r^2=0.89$), and, since no changes were found for *Rhytidiadelphus*, no correction was applied (figures 5.3 and 5.4). The equations of these lines were used to calculate tissue N and P contents adjusted for the day degrees at the time of the visit.

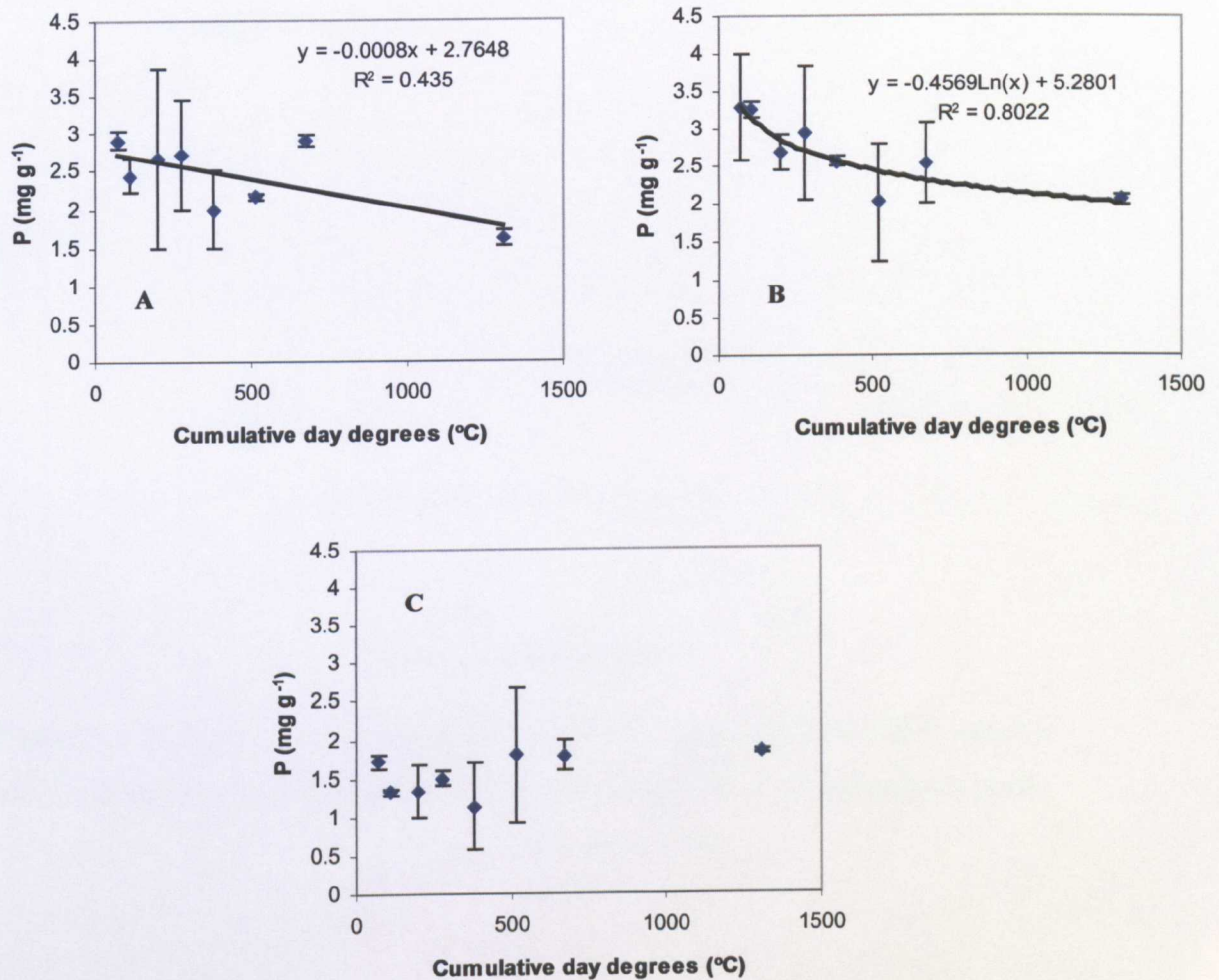


Figure 5.3 Stockgrove tissue P content (mg g⁻¹) for **A.** *A. capillaris*, **B.** *G. saxatile* and **C.** *R. squarrosus* showing equation that gives best fit.

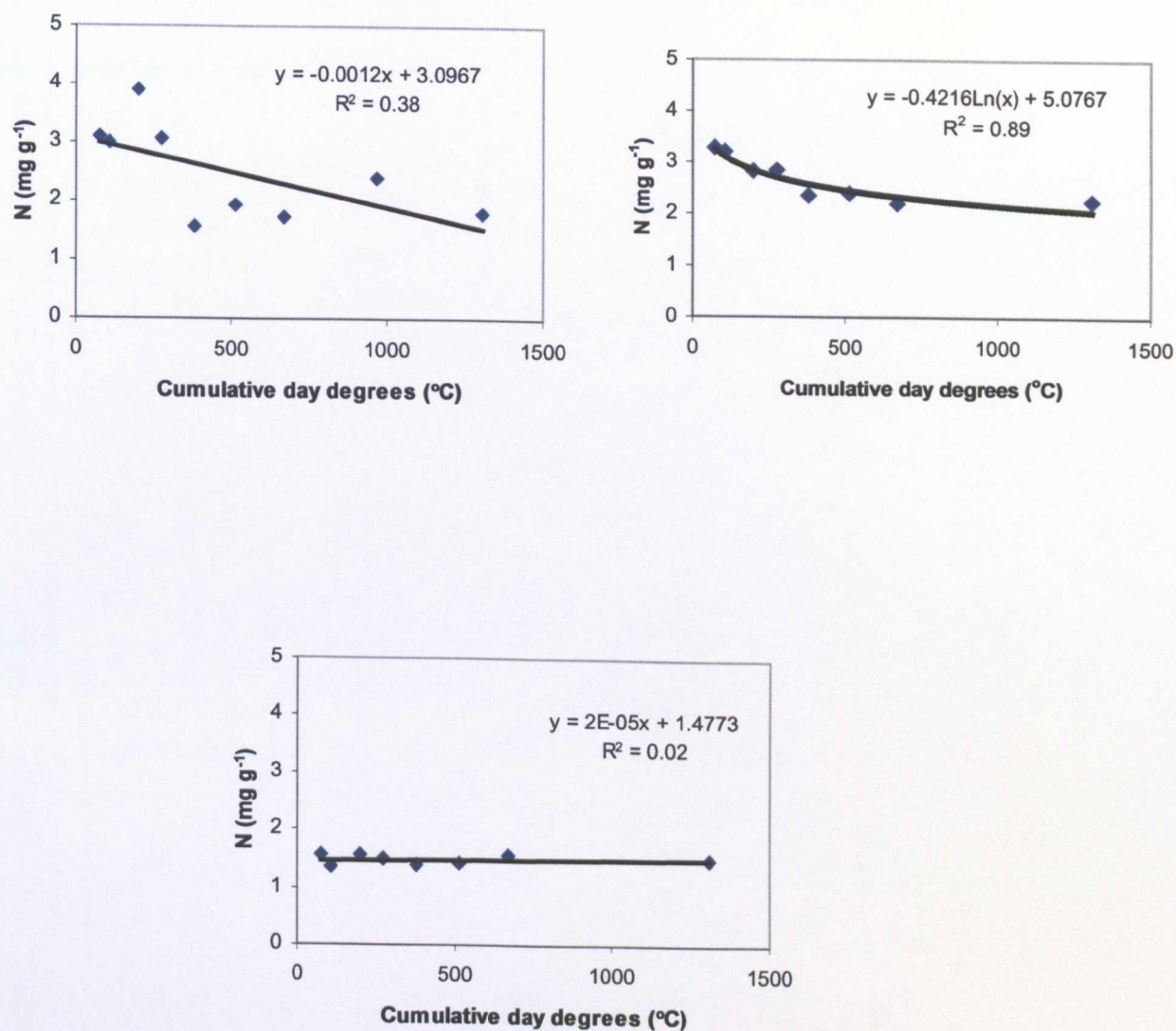


Figure 5.4 Stockgrove tissue N content (mg g⁻¹) for A) *A. capillaris*, B) *G. saxatile* and C) *R. squarrosus* showing equation that gives best fit. Limited analysis meant that error bars can not be given.

5.2.4. Data analysis

Regression analysis and multiple regression were used as described in section 3.2.2. For plant tissue chemistry the dependant variables tested were tissue N and N:P ratio of each of the three species. The independent predictor variables used were five meteorology variables (MAP, MAT, AE, PE, SMD), nine deposition chemistry variables (N dep, NH_x dep, NO_x dep, wet dep NO₃, wet dep NH₄⁺, dry dep NO₂⁻, dry

dep NH_3 , acid dep and S dep) and six environmental variables (altitude, litter, slope, aspect, grazing and enclosure) (see table 3.1).

5.3 Results

5.3.1 Tissue N

5.3.1.1 *Galium saxatile*

There is no significant relationship between total N deposition and either tissue N content or tissue N content corrected for day degrees ($p=0.15$ and $p=0.09$ respectively) (figure 5.5).

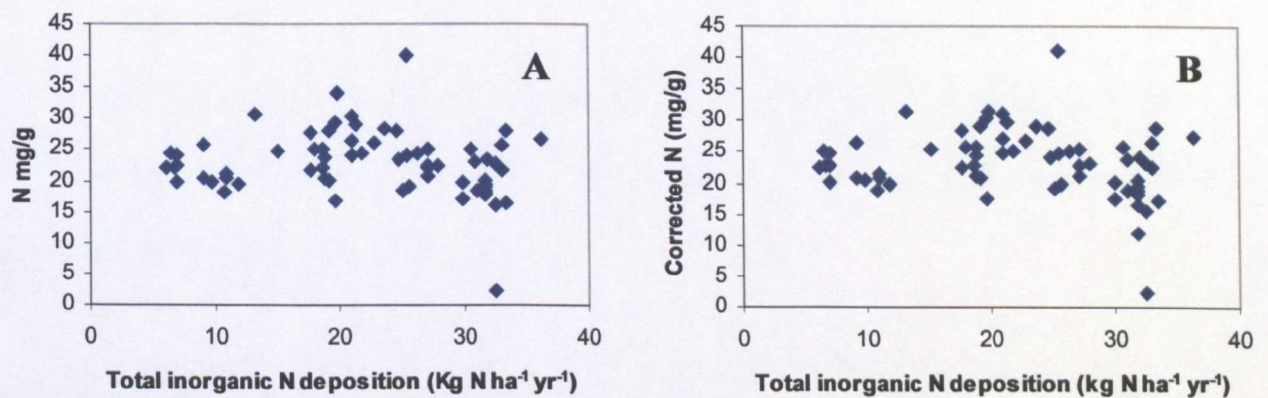


Figure 5.5 A. Tissue N content and B. Tissue N content corrected for day degrees versus total inorganic N deposition for *Galium saxatile*.

Stepwise multiple regression using environmental and soil variables (table 3.1 plus pH, soil C, N, S and P) shows that the most important factor in relation to tissue N content is the soil C:N ratio. This is a negative correlation *i.e.* as the C:N ratio increases the tissue N content decreases. 18% of the variation in tissue N is explained by the C:N ratio (figure 5.6). Temperature ($TEMP$, °C) and soil moisture deficit (SMD , mm) were the second and third most powerful explanatory variables in the regression equation (equation 5.1).

$$\text{Tissue N content} = 47.54 - 0.37(C:N) - 2.42(TEMP) + 0.07(SMD)$$

$$(R^2 = 0.31, p < 0.01)$$

(Eq. 5.1)

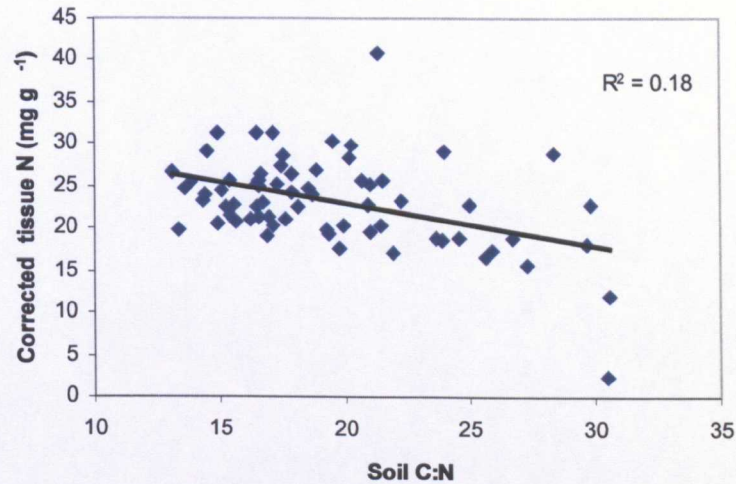


Figure 5.6 Tissue N content (mg g⁻¹) for *Galium saxatile* versus soil C:N

Using tissue N corrected for day degrees increases the correlation with soil C:N and allows more of the variation to be explained (equation 5.2), although temperature still occurs in the regression equation.

$$\text{Tissue N (corrected for day degrees)} = 50.02 - 0.46(C:N) - 2.50(TEMP) + 0.07(SMD) \\ (R^2 = 0.35, p < 0.01) \quad (\text{Eq. 5.2})$$

5.3.1.2 *Agrostis capillaris*

There is no significant relationship between total N deposition and either tissue N content or tissue N content corrected for day degrees ($p=0.12$ and $p=0.09$ respectively) (figure 5.7). Stepwise multiple regression shows that temperature followed by presence or absence of enclosure (*ENC*, enclosed or not enclosed) and subsoil pH (*SUB*) are important in relation to tissue N (equation 5.3).

$$\text{Tissue N content} = 13.01 - 1.14(TEMP) + 2.71(ENC) - 2.20(SUB) \\ (R^2 = 0.23, p < 0.05) \quad (\text{Eq. 5.3})$$

Using tissue N corrected for day degrees does not improve the model.

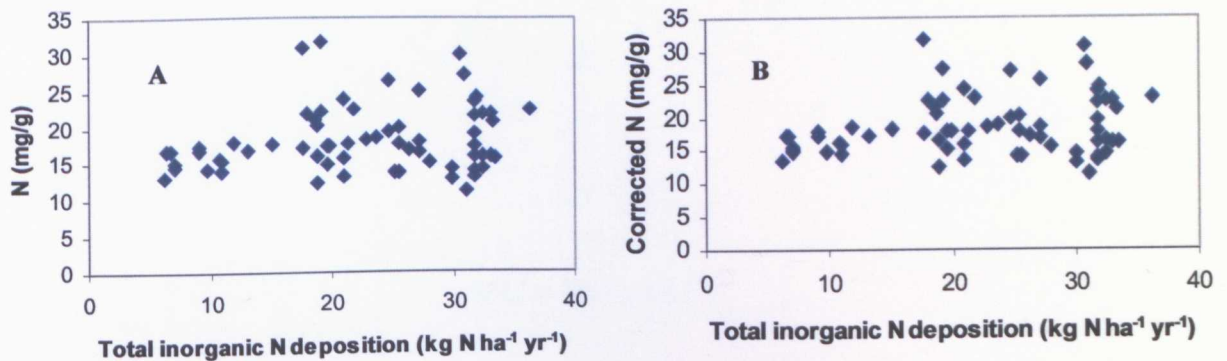


Figure 5.7 Total inorganic N deposition versus **A.** tissue N (mg g^{-1}) and **B.** tissue N corrected for day degrees (mg g^{-1}) for *A. capillaris*.

5.3.1.3 *Rhytidiadelphus squarrosus*

There was also no significant correlation between *Rhytidiadelphus* tissue N and total inorganic N deposition ($p=0.07$) (figure 5.8). Stepwise multiple regression showed that none of the variables measured showed a significant correlation with tissue N.

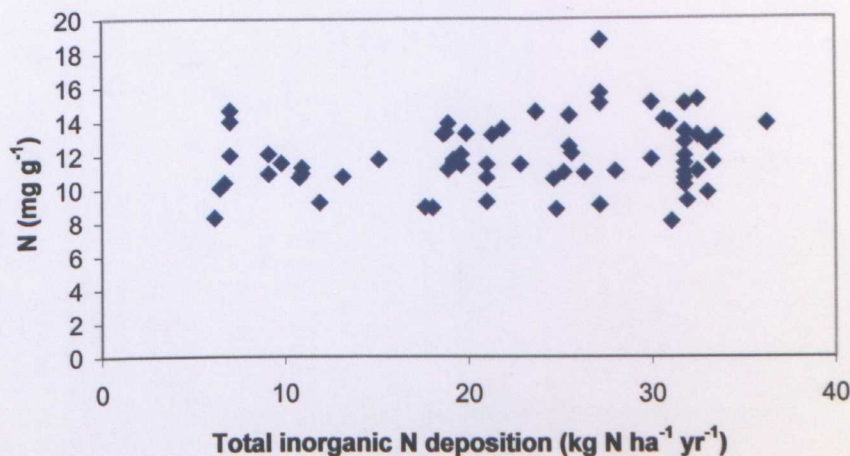


Figure 5.8 Tissue N (mg g^{-1}) versus total inorganic N deposition for *R. squarrosus*.

For all three species there are also no significant relationships with soil $\text{NO}_3^- + \text{NH}_4^+$ (KCl extractable) (figure 5.9).

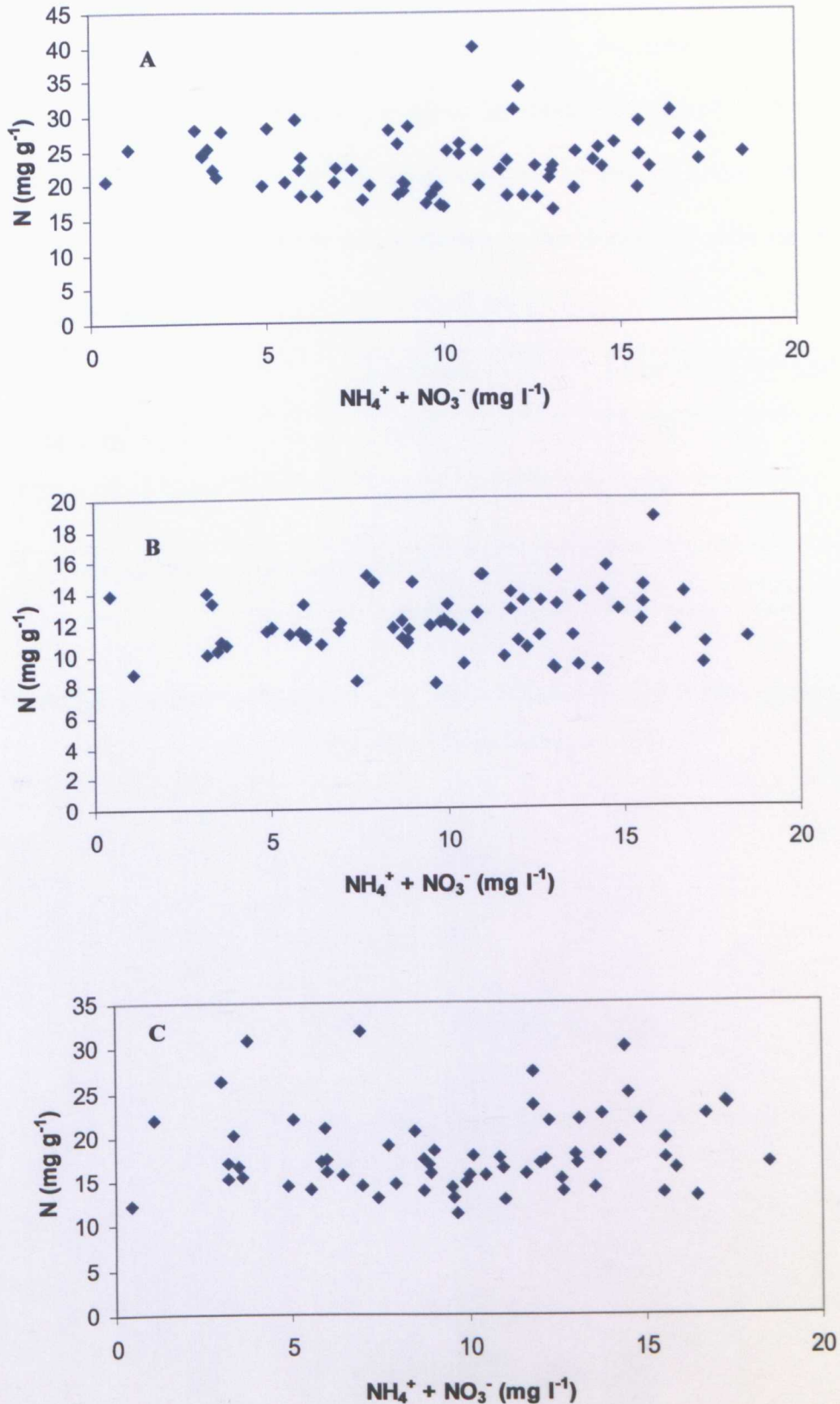


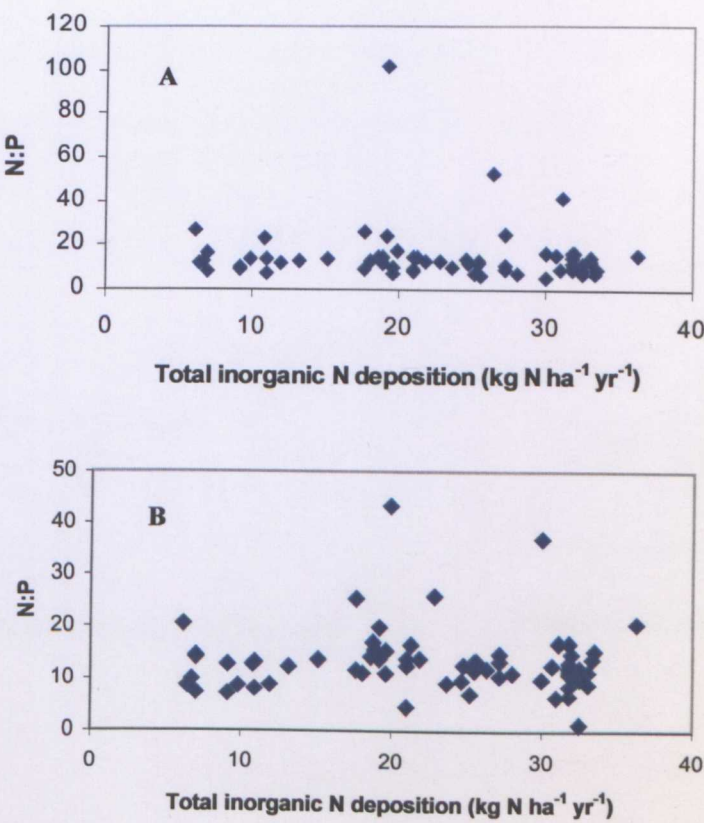
Figure 5.9 Tissue N (mg g⁻¹) versus KCl extractable NH₄⁺ + NO₃⁻ (mg l⁻¹) for **A.** *G. saxatile*, **B.** *A. capillaris* and **C.** *R. squarrosus*.

5.3.2. Tissue N:P

None of the species showed any relationship between the tissue N:P ratio and total inorganic N deposition (p values are given in table 5.2) (figure 5.10). Multiple regressions showed that none of the environmental or soil variables (table 3.1 plus pH, soil C, N, C:N and P) explained variation in the tissue N:P ratio for any of the species examined.

	N:P ratio	Corrected N:P ratio
<i>Agrostis capillaris</i>	0.69	0.78
<i>Galium saxatile</i>	0.97	0.95
<i>Rhytidiadelphus squarrosus</i>	0.92	--

Table 5.2 p values for regression analysis of plant tissue N:P ratio against total inorganic N deposition.



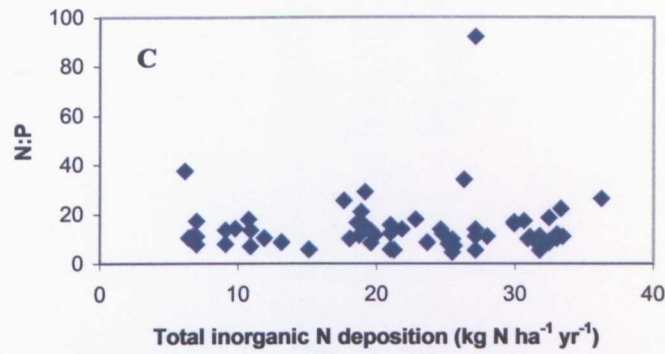
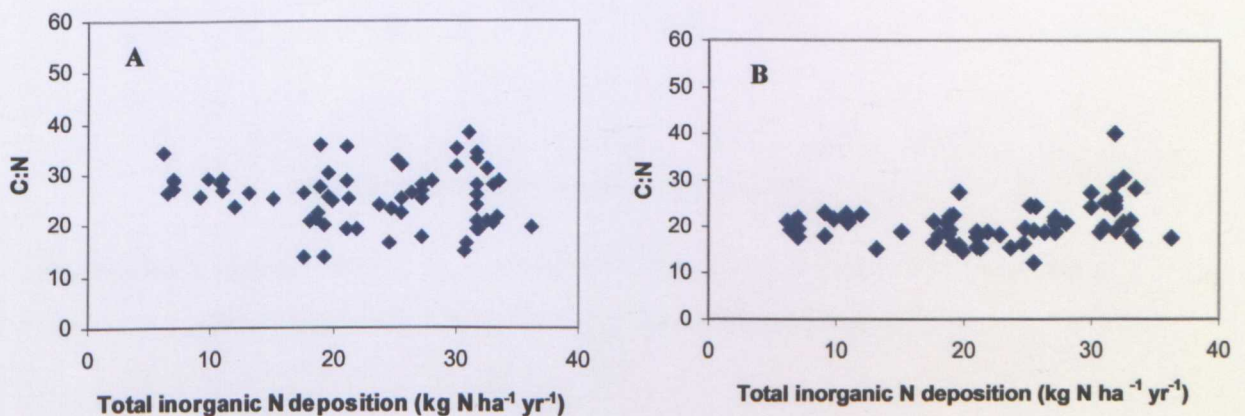


Figure 5.10 Tissue N:P ratio versus total inorganic N deposition (kg N ha⁻¹ yr⁻¹) for **A.** *A. capillaris* **B.** *G. saxatile* and **C.** *R. squarrosus*.

5.3.3. Tissue C:N

There are no significant relationships between total inorganic N deposition and the tissue C:N ratio of any of the species examined (figure 5.11). In *Galium* there does appear to be a slight increase in tissue C:N above a threshold of approximately 27 kg N ha⁻¹ yr⁻¹, opposite to what might be expected in response to higher N deposition. However, using a t-test to look at these as two groups (< 27 kg N ha⁻¹ yr⁻¹ and > 27 kg N ha⁻¹ yr⁻¹) does not give a significant difference ($t = -18.04$).



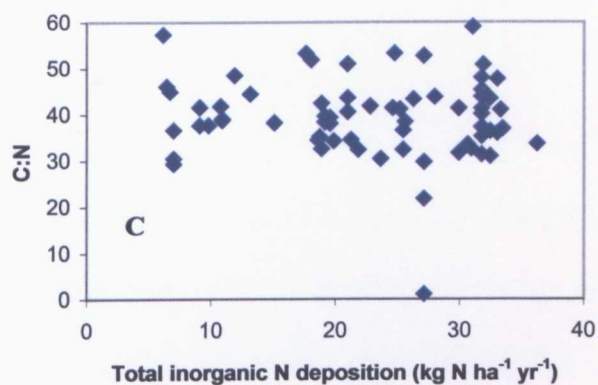


Figure 5.11 Tissue C:N ratio versus total inorganic N deposition (kg N ha⁻¹ yr⁻¹) for **A.** *A. capillaris* **B.** *G. saxatile* and **C.** *R. squarrosus*.

5.3.4. Mineralization and tissue nutrients

There is no significant relationship between mineralizable ammonium and the tissue N content of any of the species examined (figure 5.12).

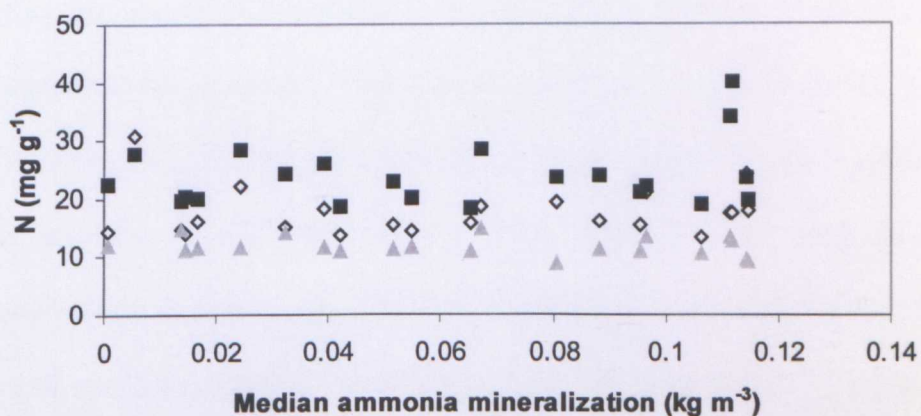


Figure 5.12 Median ammonia mineralization (kg m⁻³) and tissue N content (mg g⁻¹) for *A. capillaris* (◇), *G. saxatile* (■) and *R. squarrosus* (▲).

5.4 Discussion

5.4.1 Seasonal variation

Nutrient concentrations in above-ground biomass have been shown to decrease during the growing season (Chapin *et al.*, 1980; Gusewell and Koerselman, 2002). However, in grazed grasslands this is not always the case, as late-summer re-growth may have enhanced nutrient concentrations (Olff *et al.*, 1994). Despite intensive grazing by rabbits at Stockgrove Country Park, tissue N and P for both *Agrostis capillaris* and *Galium saxatile* declined over the summer. There was no increase in the nutrient content to signify a late-summer re-growth, which may be because the grazing is persistent and there is little opportunity for re-growth in late-summer.

Marrs (1978) found nitrogen concentrations in ericaceous species tended to be lowest during the spring and highest at the end of the growing season in late summer. Phosphorus concentration tended to be higher in the early spring prior to shoot elongation, and then declined throughout the growing season. In this investigation this was the pattern identified for phosphorus in both *Agrostis* and *Galium*. *Rhytidadelphus squarrosus* showed little variation in the tissue N or P concentration. Powell and Malcolm (1974) show that different components of a grassland system show different seasonal variations in biomass N and P, with *Calluna vulgaris* even showing variation during different growth stages. Variation in the P concentrations and to a lesser extent N concentrations from year-to-year have also been identified, and attributed to climatic variation (Powell and Malcolm, 1974).

An attempt was made to correct for this seasonal variation in the N and N:P ratios using day degrees. Despite this correction climatic variables were still important factors in some of the multiple regressions, indicating that climatic differences between the sites were not entirely removed by the correction. The use of long-term averages meant that year to year variation was lost, which may have been an important factor. Temperature data were also only available on a large scale and consequently were not very precise spatially. Refined estimates using temperature data modelled by the MET Office could be made on a site by site basis, but this was prohibitively expensive for the present study, and further investigation is required.

5.4.2 Tissue N

None of the three species examined showed a significant correlation between N deposition and tissue N content. Many other studies, particularly experimental N additions, have shown correlations between the level of N addition and the tissue N content. Increases in tissue N in relation to experimental N application have been shown in *Calluna vulgaris* (Carroll *et al.*, 1999), *Vaccinium vitis-idaea*, *Erica cinerea*, *Eriophorum vaginatum* (Leith *et al.*, 1999), mosses (Pitcairn *et al.*, 1998, Gordon *et al.*, 2001), pine and hardwood tree species (Magill *et al.*, 1997) and some grasses and forbs (Morecroft *et al.*, 1994, Carroll *et al.*, 2003). The majority of these N applications were made at levels above those realistically seen in the UK, or results were only detected at such levels. However applications of $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ caused increase in the tissue N content of *Dicranum scoparium* and *Polytrichum juniperinum* in high Arctic heath where background levels are $1\text{--}10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Gordon *et al.*, 2001).

Several experimental additions have failed to show increases in tissue N with N addition. Uren *et al.* (1997) found significant differences in the shoot N content of *Calluna vulgaris* at application rates of 7.7 and 15.4 kg N ha⁻¹ yr⁻¹ only in the first year of addition. No statistically significant results were found in the subsequent 3 years of the experiment. Marcos *et al.* (2003) suggest that using low levels of N addition cannot be expected to produce a large N increase in the vegetation. Pearce and van der Wal (2002) found no increase in tissue N content of *Racomitrium lanuginosum* by the end of the growing season in which N was applied. These results suggest that, although plants can take up excess N in these systems they are able to use it rapidly for increased growth. Biomass measurements would provide confirmation of this explanation and are a particularly important area of future study. In N-limited systems, this would be expected as additional N is used rapidly in growth rather than being stored as a result of luxury uptake.

A number of studies have demonstrated correlations between N inputs in atmospheric deposition and the foliar N content of vegetation including conifer needles (Dise *et al.*, 1998) (see section 1.1.2.1), *Calluna vulgaris* (Pitcairn *et al.*, 1995, Sanger *et al.*, 1996, Hicks *et al.*, 2000), and *Nardus stricta*, *Deschampsia flexuosa*, *Erica cinerea* and *Hylocomium splendens* (Hicks *et al.*, 2000). Kirkham (2001) found no correlation between N content and NH_x deposition but did find a relationship between NO_x deposition and *Calluna* and *Agrostis/fescue* tissue N content. Other species did not show significant relationships.

Soil C:N was the factor most closely related to tissue N in *Galium saxatile*. The fact that it is the C:N ratio rather than soil N that shows this relationship, demonstrates the

importance for the plants of the form in which the N is stored in the soil. However, using KCl extractable NH_4^+ and NO_3^- as surrogates for available N does not show a trend (figure 5.5). Variability of the soil types may be an important reason that a stronger relationship between N deposition and tissue N was not found. Morecroft *et al.* (1994) identified a significant increase in tissue N in *Galium* with N inputs of 35, 70 and 140 kg N ha⁻¹ yr⁻¹, although the increase was very slight at 35 kg N ha⁻¹ yr⁻¹ (the highest level in the present study). These responses are to short term applications, whilst the data in the present study represent a long-term equilibrium. Despite the importance of climatic variables in determining tissue N content there was no difference apparent for any of the species between the samples collected in 2002 and the 8 collected in 2003. 2002 was a very wet summer compared to the warm dry summer of 2003. There was no clear relationship between tissue C:N ratio and N deposition which is likely to be due to the variability in the tissue percentage N.

Several authors have suggested tissue N content as an indicator of N deposition (Hicks *et al.*, 2000, Pitcairn *et al.*, 1995). However, the findings of the present study do not support the use of tissue N content as a suitable indicator on a national scale. Only *Galium* showed any relation between tissue N and N in the environment with a significant relationship to soil C:N. However, the relationship in these sites between soil C:N and N deposition is tenuous at best (chapter 4). Climatic variables are clearly of great importance, something that would be difficult to control for using tissue N content an indicator of N deposition and there is seasonal variation. Some species are clearly better indicators than others. Review of the literature suggests that ericaceous species, especially *Calluna vulgaris*, show the best response.

5.4.3 Tissue N:P

The results for all three species showed that the N:P ratios were not significantly related to any of the environmental variables measured. This outcome is surprising as climatic, soil and atmospheric variables were all considered. Soil available N would be expected to give a better relationship than soil total N, and one we might also expect relationships with soil available P and soil pH (Schaffers, 2002). Grazing intensity was only fairly crudely estimated, and management history in terms of past grazing was not accounted for. These are both factors that could have influenced the tissue mineral content.

Several studies have found a relationship between N:P ratios and N deposition. On a gradient of N deposition in England and Wales, Kirkham (2001) reported a significant correlation between NH_x deposition and N:P ratio in *Nardus stricta* and with NO_x deposition in *Calluna vulgaris*, *Vaccinium myrtillus* and *Nardus*. However, surveys were taken over two years and the trends were not necessarily consistent for both of the years. This result suggests that tissue N:P variation is also strongly affected by climate and hence is not a good indicator or predictor of N deposition. In this study, there was also considerable variation in the N contents of the three species along the N deposition gradient but Pearson product moment correlation coefficient showed no significant correlation between N:P ratios of the 3 species (table 5.3).

	<i>Rhytidiadelphus</i>	<i>Galium</i>	<i>Agrostis</i>
<i>Rhytidiadelphus</i>	--	p = 0.341	p = 0.300
<i>Galium</i>		--	p = 0.140
<i>Agrostis</i>			--

Table 5.3 Correlation between N:P ratios on *Agrostis capillaris*, *Galium saxatile* and *Rhytidiadelphus squarrosus* in the present study.

The N:P ratio of plant tissues has been suggested as an indicator of nutrient limitation (Koerselman and Meuleman, 1996, Gusewell and Koerselman, 2002). P limited species should have a higher N concentration and a lower P concentration and hence a higher N:P ratio than N limited or co-limited species. In this index an N:P mass ratio of greater than 16 indicates P limited vegetation, one of 13.5 – 14 co-limitation and a ratio less than 14 N-limitation (Gusewell and Koerselman, 2002). The three species indicate different limitation for different sites. Using *Agrostis*, 16% of the sites were P limited, with N deposition ranging from 7.0-31.9 kg N ha⁻¹ yr⁻¹. Using *Galium* 19% of the sites were P limited, with N deposition ranging from 6.2-36.3 kg N ha⁻¹ yr⁻¹. With *Rhytidiadelphus* 24% of the sites were P limited, with a range of 7.0-36.3 kg N ha⁻¹ yr⁻¹ with a small degree of overlap. There is no relationship between N deposition and nutrient limitation.

There were exceptions in the Gusewell and Koerselman (2002) study, which determined these thresholds for nutrient limitation, and some species were found to have large ranges in their N:P ratio. Of the three species examined in this study only *Agrostis* was investigated by Gusewell and Koerselman (2002). Many of the N:P values in this study were within the range found by Gusewell and Koerselman, though some were considerably higher. The results of a study carried out by Hofmeister *et al.* (2002) challenge the idea of using N:P ratio to indicate nutrient limitation. They found plots in the Bohemian Karst with a high incidence of nitrophilic plants, high soil N and high N deposition that, by using N:P ratios appeared to be N limited, and plots in Slovenia where few nitrophilic plants were found that were not N limited. These results, in addition to those used in this study, would suggest that the N:P ratio is not effective for the determination of N deposition. The lack of a relationship with N deposition could be partially due to the differences in the soils encountered in this

study and their capacities to hold nitrogen in different forms and their relative availability to plants. The differences in the soils would affect the nutrient limitation at the sites and may account for the lack of relationship in this study.

5.5. Summary

In summary there are no relationships between plant tissue N content and N inputs in the three species tested; consequently I would not recommend it as an indicator of N deposition. Examination of N:P ratios in tissues is inconclusive as there is a disagreement between the sites indicated as P limited by the different species. Further investigation is needed before conclusions can be drawn about the nutrient limitation of communities derived from the N:P ratios of individual species.

6. Plant-Soil interactions

6.1 Introduction

The aim of this chapter is to investigate plant-soil interactions in relation to N deposition. It will link the effects of N deposition on soils with effects that have been detected in the species richness and composition of the vegetation.

Soil characteristics such as nutrient status, pH, and metal concentrations are very important in determining the species found in grasslands. Different species have different optimal conditions for growth and hence differing abilities to compete in different soils. Consequently it is likely that the impacts of N deposition on soils are at least partially responsible for the differences in species richness and composition detected along the gradient of inorganic N deposition, in addition to direct impacts of N on the vegetation. This chapter aims to identify direct and indirect (via soil) effects of N deposition on species richness and composition (presence and abundance of species).

6.2 Methods

6.2.1 Field methods

Plant species data, subsoil and topsoil samples and samples of plant tissues from 3 species (*Agrostis capillaris*, *Galium saxatile* and *Rhytidiadelphus squarrosus*) were collected from 68 randomly selected U4 grasslands along a gradient of nitrogen deposition as described in section 2.3. Modelled values for N deposition were provided by the Centre for Ecology and Hydrology (CEH), Edinburgh (section 2.3).

6.2.2 Laboratory methods

Soils were analysed for pH, carbon and nitrogen content (%), KCl extractable ammonium and nitrate concentration (mg l^{-1}), KCl extractable metals (ppm) and plant available P (mg kg^{-1} - Olsen). Methods are described in section 4.2. Plant tissue samples were analysed for C, N and P (mg g^{-1}) as described in section 5.2.

6.2.3 Data analysis

Forward stepwise multiple regression was carried out (discussed in section 3.2.2) with species richness as the dependant variable. Non-soil environmental variables, as well as soil and plant-tissue variables were used in the stepwise multiple regression. Canonical correspondence analysis (CCA) was carried out as discussed in section 3.2.3. Species are ordinated within a multi-dimensional ordination space according to their presence and abundance. Associations between extremes for environmental variables (e.g. high N deposition) were identified by looking for species consistently located at high or low values for those variables. Arrows on the ordination diagram represent the environmental variables. To reduce multi-collinearity in the CCA,

selected environmental variables (altitude, aspect, enclosure, grazing, total inorganic N deposition, total S deposition, slope and soil moisture deficit - section 3.3.2) were used together with soil variables. Litter cover, subsoil pH, topsoil pH, soil % C, % N and C:N ratio were also used (chapter 4). For KCl extractable metal (concentrations of aluminium, calcium, chromium, copper, iron, lithium, magnesium, manganese, nickel, silicon, sodium and zinc) subsoil samples only were used, as there was close correlation between topsoil and subsoil metal concentrations (see appendix 6). *Galium saxatile* was used to represent the plant tissues for tissue N and N:P ratio.

6.3 Results

Of the 36 environmental, soil and plant variables tested, total inorganic N deposition showed the strongest correlation with species richness ($r^2 = 0.55$, $p < 0.01$, chapter 2). Mean annual precipitation explained an additional 7% variation, followed by subsoil calcium concentration explaining a further 7% variation, and altitude accounting for 3%. Subsoil lithium concentration explains a final 3% variation, giving a total R^2 of 0.77:

Species richness = 14.88 − 1.57 (*Ndep*) + 0.04 (*MAP*) + 0.03 (*Calcium*) − 0.009 (*Alt*) +
2.61 (*Lithium*)

($R^2 = 0.77$, $p < 0.01$, $n = 68$)
(Equation 6.1)

Using all 36 variables, forward selection in the canonical correspondence analysis shows that total inorganic N deposition remains the most important variable for explaining variation in the distribution of species. This is followed by lithium concentration, subsoil pH, altitude, lead concentration and soil moisture deficit. Table 6.1 shows all variables in order of the amount of variance they explain singly (i.e. if used as the only environmental variable) with regard to the distribution of species.

Variable	Lambda-1
N deposition	0.1
Mean annual precipitation	0.09
Subsoil lithium concentration	0.09
Mean annual temperature	0.08
Potential evapotranspiration	0.07
Subsoil pH	0.07
Altitude	0.07
Subsoil lead concentration	0.06
Actual evapotranspiration	0.06
Soil moisture deficit	0.06
Subsoil zinc concentration	0.06
Subsoil barium concentration	0.06
Topsoil pH	0.06
Grazing	0.06
Subsoil iron concentration	0.06
Subsoil chromium concentration	0.05

Nickel concentration	0.05
Subsoil copper concentration	0.05
Topsoil C:N ratio	0.05
Subsoil silicon concentration	0.05
Subsoil sodium concentration	0.04
Slope	0.04
Litter cover	0.04
<i>G. saxatile</i> tissue N	0.04
Subsoil arsenic concentration	0.04
<i>G. saxatile</i> tissue N:P	0.04
S deposition	0.04
Subsoil manganese concentration	0.03
Topsoil % N	0.03
Topsoil % C	0.03
Enclosure	0.03
Aspect	0.03
Subsoil aluminium concentration	0.03
Topsoil plant available phosphorous	0.02
Subsoil magnesium concentration	0.02
Subsoil calcium concentration	0.02

Table 6.1 Variables in order of their degree of correlation with regard to the distribution of species as given by CCA forward selection. Lambda value represents amount of variation explained.

Ordination diagrams were created using selected environmental variables to reduce multi-collinearity. Examination of the environmental, soil, and plant variables shows that some are correlated with each other in the 3-dimensional ordination space (figure 6.1). High mean number of species is consistently positively correlated with high subsoil and topsoil pH and high subsoil sodium concentration. It is also correlated with low N deposition, low soil moisture deficit, low subsoil aluminium concentration and low S deposition. High soil N concentration is correlated with high soil C and subsoil arsenic concentration. C:N ratio and altitude are also positively correlated. It is important to remember that only two axes from a multidimensional ordination space are represented here (figure 6.1). Correlations are identified by examination of several axis combinations.

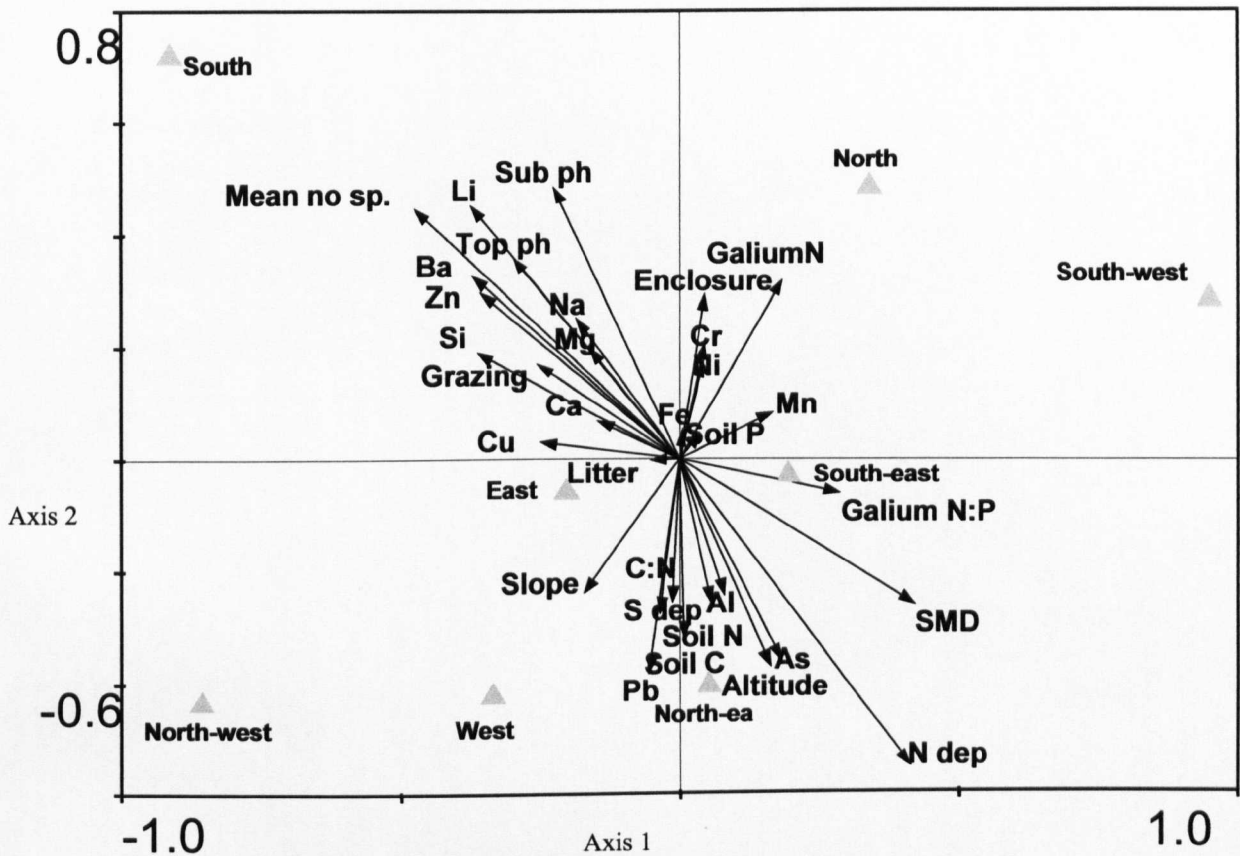


Figure 6.1 CCA ordination showing selected environmental, soil and plant variables.

The species biplot (figure 6.2) shows that combining soil and environmental variables explains a larger degree of variation in the overall species composition than environmental variables alone (figure 3.5) (species-environment correlation for the first axis: environmental + soil = 0.929; environmental only 0.786). The same species are positively and negatively associated with high N deposition as those identified in chapter 3 (section 3.3.2), however, one can make an additional examination of the correlation of species with soil variables.

There are no species for which abundance is correlated with topsoil % soil N. Mat grass (*Nardus stricta*) appears to be positively correlated with soil C:N ratio. There are also several species correlated with topsoil P. *Polytrichum commune* and to a lesser extent *Rhytidiadelphus squarrosus* and yarrow (*Achillea millifolium*) all show a

positive correlation, whereas heath rush (*Juncus squarrosus*) and wavy hair grass (*Deschampsia flexuosa*) are correlated with low P. In general topsoil P does not exert a strong influence over the distribution of species, as shown in figure 6.2 by the comparatively short arrow. *Dicranum scoparium* and pill sedge (*Carex pilulifera*) appear to be correlated with a high tissue N:P ratio in *Galium saxatile*.

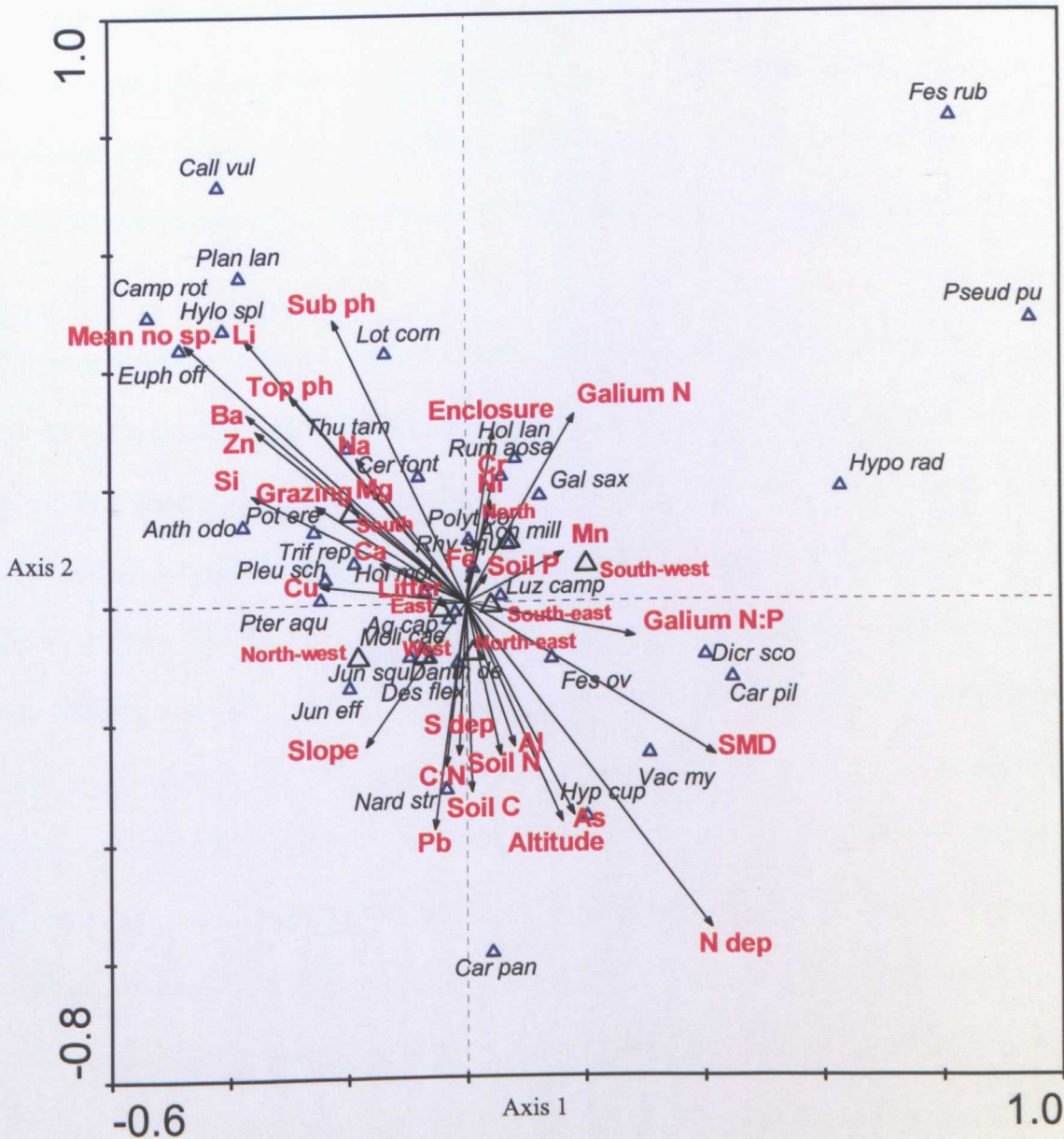


Figure 6.2 CCA ordination showing species, soil and environmental variables. Δ represents discrete environmental variables; \rightarrow represents continuous environmental variables with arrows pointing in the direction of increasing values.

6.4 Discussion

6.4.1 Plant – soil interactions

The overriding importance of total inorganic N deposition to species richness is demonstrated by equation 6.1. N deposition explains 55% of the variation in species richness, confirming the findings of chapter 3. 35 variables were included in the stepwise multiple regression, including environmental, soil variables and plant tissue variables for *Galium saxatile*. Of these only 4 variables in addition to N deposition were selected, to give a total of 77% of the variation in species richness explained.

Reasons for the decline in species richness that can be attributed to N deposition are discussed in section 3.4.3. Rainfall and altitude, also discussed in that section, explain additional variation. Subsoil calcium (Ca^{2+}) concentration is the third most important explanatory variable to emerge from the regression analysis. It is likely that this result reflects soil pH to some extent, as calcium concentration is important in determining soil pH, and Ca^{2+} availability is also strongly determined by subsoil calcium (Cresser *et al.*, 1993). In the present analysis subsoil calcium was indeed related to pH (table 4.1) and topsoil and subsoil calcium were significantly correlated. Diversity is generally higher in higher pH soils (Partel, 2002). As the relationship between diversity (or species richness), and pH is not strong in this study, it is unlikely to be the sole reason for the relationship between species richness and subsoil calcium. Soil calcium and to a lesser extent pH are internal soil properties, due to the geology, climate or management. These factors modify the effects of N deposition, an additive effect also observed in the Park Grass experiment (Silvertown, 1980).

The importance of calcium may also represent the soil's capacity to buffer against changes in the pH of the soil, brought about by nitrogen deposition. Soils rich in calcium and other basic cations are able to exchange these cations for hydrogen ions and hence are better buffered against acid inputs. Small changes in Ca^{2+} activity can affect the concentrations of other plant nutrients and potentially toxic metals (Rorison and Robinson, 1984). A soil in which pH and Ca^{2+} concentration have declined could potentially have a lower diversity than one that has remained stable, with the pH change being the important factor rather than the actual pH value itself.

Beyond its buffering influence on soils, calcium is also essential for plant growth (Cresser *et al.*, 1993); this could further explain its importance to species richness. Plants from soils low in Ca^{2+} have evolved lower requirements for and a lower tolerance of Ca^{2+} levels. It is thought that the influence of soil pH upon plant species distribution is mediated through indirect effects including Ca^{2+} availability (Rorison and Robinson, 1984).

The positive correlation with lithium is interesting. Subsoil lithium concentration is highly correlated with both topsoil and subsoil pH (table 4.1) and thus this result may arise from a simple autocorrelation in the regression equation. Lithium is not known to be an essential plant nutrient (Kabata-Pendias and Pendias, 1992), although there is some evidence that it can promote plant growth and development, possibly being used in enzyme activation in place of potassium. There is considerable difference in the tolerance of various species to lithium concentration (Angino *et al.*, 1974).

As with species richness determined by multiple regression, forward selection by CCA shows that, of the 36 variables considered, N deposition is also the most important variable in determining the distribution of species (table 6.1). N deposition is followed in this respect by mean annual precipitation. Interestingly lithium is again very important, coming third in the list of variables, followed by potential evapotranspiration and subsoil pH, altitude and lead.

The importance of N deposition to species distribution is likely to be a result of a combination of eutrophication and acidification effects. Eutrophication should lead to an increase in the number and abundance of species that are either nitrogen-demanding or able to take advantage of the additional available nitrogen, such plants outcompete other species, eventually reducing richness. Acidification should lead to an increase in the strongly calcifuge species and a decline in calcicolous species. Climate is clearly very important in determining the distribution of species; not only is rainfall the second most important variable in the CCA forward selection output, but all five climate variables (rainfall, temperature, actual and potential evapotranspiration and soil moisture deficit) occur within the first 10 variables. These variables have already being discussed above and in chapter 3.

It is not surprising to see that soil pH is important for species distribution. Soils with a lower pH would be expected to have a greater abundance of acid-tolerant species and fewer species more typical of circumneutral environments. This variable is followed in importance by altitude, which is most likely to be important to the distribution of species through its correlation with climatic variables. Lead

concentration is closely related to soil pH (see chapter 4) and is also toxic to plants (Kabata-Pendias and Pendias, 1992).

The variables important in the distribution of species are very similar to those identified in the multiple regression as important for species richness. Subsoil calcium is the exception to this and despite being very important for species richness, coming out third in the stepwise multiple regression, it is last in the CCA forward selection.

Examination of the ordination of the environmental variables within the CCA plot (figure 6.1) demonstrates relationships between variables. A high mean number of species is positively correlated with both subsoil and topsoil pH, supporting the hypothesis that the occurrence of calcium and lithium in the multiple regression may be a reflection of changes in soil pH. Many studies have identified positive correlations between soil pH and species diversity (e.g. Partel, 2002; diversity and richness closely correlated in this study – $r=0.86$) and species richness has been found to be lowest at a pH of less than 5 (Critchley *et al.*, 2002). Roem *et al.* (2002) identified acidification as the most important factor in reducing species richness in heathlands in a study where nutrient availability and soil pH were manipulated independently.

There is a negative relationship between species richness and soil moisture deficit. (figure 6.1) and S deposition is also correlated with N deposition. These variables are not shown to be important in the regression analysis because they do not explain additional variation to that explained by N deposition. The correlation with sulphur

highlights the fact that the high correlation between species richness and N deposition may have a component of a relationship with S deposition included that is masked by the greater correlation with N deposition.

The negative relationship between species richness and reactive aluminium (Al^{3+}) concentration of the soil is not surprising as aluminium is mobilised with reduced pH (see chapter 4). This process is then reflected in a reduced species richness because aluminium is toxic to plants. Once mobile in acid solution, Al^{3+} can be taken up by plants, creating chemical stress (Kabata-Pendias and Pendias, 1992). With the exception of soils with a very high organic matter content (where aluminium is bound to organic matter), below a pH of 5.5 soils may contain increasing concentrations of aluminium ions that may in turn affect the vegetation composition.

Aluminium is released into solution at a pH of approximately 5 as H^+ replaces it on colloidal exchange sites (Ashman and Puri, 2002). Even at very low soil solution concentrations (10^{-6} M), sensitive plants can be harmed (Wild, 1993) by inhibiting cell division and elongation of plant roots (Ashman and Puri, 2002). Plants that are indigenous to acidic semi-natural grasslands possess a number of adaptive features to enable them to survive at low pH (Higgs and James, 1969) and tolerate aluminium stress (Feng Ma *et al.*, 2001). Some races of species that are widespread in these environments, such as *Holcus lanatus*, may show tolerance to H^+ and Al^{3+} (Kidd and Procter, 2001).

Aluminium toxicity brought about by acidification has been identified as the factor most important to species richness in heathlands in the Netherlands (Roem *et al.*,

2002). In germination tests, a dissolved aluminium treatment ($28.1 \text{ g Al}^{3+} \text{ m}^{-2} \text{ yr}^{-1}$) reduced the germination of heathland species to almost zero. Foy (1983) reports Al toxicity in subsoils as particularly harmful because it causes shallow rooting, drought susceptibility and poor use of soil nutrients. Higher organic matter content of topsoils is also an important factor, which is reflected in this investigation in the lack of a significant correlation between species richness and aluminium concentration in topsoils ($r^2 = 0.04$, $p = 0.13$) as opposed to the significant negative relationship in subsoils ($r^2 = 0.19$, $p < 0.001$) (see chapter 4).

Figure 6.1 shows the expected positive correlations between topsoil N and topsoil C (and C:N ratio). More interesting are the correlations between these variables and altitude. The correlation with altitude may be a climatic relationship, as sites at high altitude tend to receive more rain and have lower temperatures, with consequent slow breakdown of organic matter.

The species biplot (figure 6.2) shows correlations between species and N deposition (and correlated variables as discussed in chapter 3), and allows examination of correlations with soil variables. Total inorganic N deposition and pH are too closely correlated with each other to be able to separate the effects of soil acidification and N deposition. Differentiating these would require a factorial experiment as carried out by Roem *et al.* (2002).

No species were associated with high or low soil N (%), probably because total N was used rather than plant available N. Using total N means that nitrogen tied up in organic material, immobilised by microbes or otherwise unavailable to plants is

included in the analysis. Soils with a large proportion of organic material have a high total nitrogen content, but as the plants cannot utilise all of this, the total nitrogen content of these soils may be of little significance to the plant.

Soil P content is not important in determining the overall distribution of plant species, backing the postulation in chapter 5 that the majority of the sites are N limited. Nevertheless, soil P content seemed to have an important influence over some species, since phosphorus is an important nutrient for plant growth. *Polytrichum commune*, *Rhytidiadelphus squarrosus* and *Achillea millefolium* abundance all show a positive correlation with soil P. *Juncus squarrosus* and *Deschampsia flexuosa* are negatively correlated to P and both are species with low nutrient requirements (Hill *et al.*, 1999).

The observation in this study that soil P content does not exert a strong influence over the distribution of species (figure 6.2) and that soil P content is unrelated to N deposition (section 4.3.4) are interesting. Several studies, working on different types of grassland, have demonstrated that soil P is an important variable for species richness (Critchley *et al.*, 2002) and species abundance (McCrea *et al.*, 2001). Experimental applications of low levels of P ($5 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) have been shown to increase bryophyte cover in arctic tundra, suggesting that in some habitats P can be important in promoting bryophyte growth (Gordon *et al.*, 2001).

Dicranum scoparium and *Carex pilulifera* are correlated with a high N:P ratio in *Galium saxatile* tissue, which could be an indication that they are associated with P limitation. High N:P scores have been identified as an indicator of nutrient limitation (Koerselman and Meuleman, 1996, Gusewell and Koerselman, 2002) as discussed in

section 5.4.3, and P limitation could favour certain species. There is little research on mosses in relation to nutrient limitation. However, *C. pilulifera* is a species in which phosphate has been shown to limit growth in some situations, which in turn resulted in low tissue concentrations of phosphorus (Tyler, 1996). Research investigating nutrient limitation on species of calcareous grasslands has indicated some *Carex* species are advantaged by P limitation (Phoenix *et al.*, 2003a). However, further work is needed to establish which nutrients are limiting at acid grassland sites of the type included in the present research.

6.4.2 Indicators of N deposition

As mentioned in chapter 5, one of the currently favoured ideas for an indicator of levels of N deposition and ecosystem health (the condition of the land and its wildlife, Thompson *et al.*, 2004) in response to N deposition is tissue N content, particularly of *Calluna*, a species which has received considerable attention (e.g. Carroll *et al.*, 1999). Gregory *et al.* (2004) describe 4 types of indicators (figure 6.3). The first (type 1) indicators look at how specific taxa are changing. Such indicators have a weak ability to generalise regarding the response of a community as a whole and a weak link to the driving force causing the change. Type 2 indicators have a weak link to the driver and a strong ability to generalise; an example might be looking at biodiversity generally rather than in relation to a driver. Type 3 indicators, of which tissue N content may be an example, have a strong link to the driving force and a weak ability to generalise. The final grouping (type 4) has a strong ability to generalise and a strong link to the driver and includes the response of biodiversity to a driver. Using species richness as an indicator of total inorganic N deposition in U4 grasslands falls into this category. To determine whether species richness can be used

as a more general indicator of N deposition would require further research to see if U4 is typical in its response to N deposition in relation to other habitats and the wider landscape.

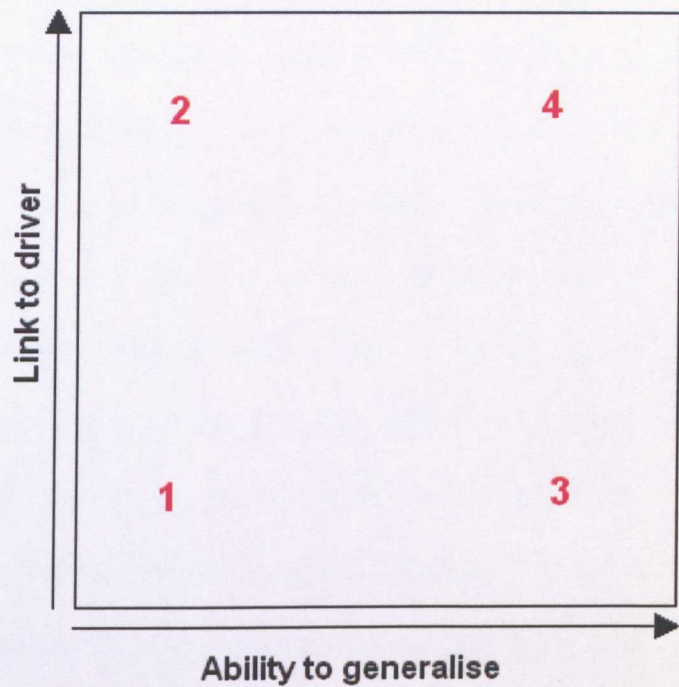


Figure 6.3 Types of indicators (after Gregory *et al.*, 2004).

Gregory *et al.* (2004) also outline a number of statistical and practical requirements of indicators (table 6.2).

Indicator properties
Quantitative
Simplifying
User-driven
Policy-relevant
Scientifically credible
Responsive to change
Easily understood
Realistic to collect
Susceptible to analysis
Cost-effective
Easily updated

Table 6.2 Statistical and practical requirements of indicators (after Gregory *et al.*, 2004).

With an r^2 of 0.55, species richness is not a good predictor of N deposition, but plants provide excellent indications of the response of the system as a whole to pollutant inputs. Using species richness as an indicator of ecosystem health in response to N deposition fulfils the majority of criteria in table 6.2 and gives quantitative outputs. Experimental N applications (e.g. Mountford *et al.*, 1993) have shown species richness is responsive to changed N availability. Species richness is susceptible to analysis because it is a simple count and, when measured per given area, it is a scientifically credible measure of the number of species present. The present study demonstrated that species richness is also related to N deposition. The concept of species richness is very easy to understand and both easy and realistic to collect, making it cost effective and user-driven. Its simplicity makes it readily updated and, when focussing on a single community (e.g. U4 grassland), it simplifies large amounts of data regarding the species composition. As biodiversity is considered so important (e.g. the 2010 biodiversity target devised by the Secretariat of the Convention on Biological Diversity), using species richness as such an indicator is extremely policy relevant.

6.5. Summary

N deposition is clearly the most important factor in explaining variation in both species richness (equation 6.1) and species composition (table 6.1). Together with subsoil calcium concentration, altitude and subsoil lithium concentration, 77% of the variation in species richness is explained. In the ordination diagram (figure 6.1) we can also relate species richness to pH, climate (soil moisture deficit), S deposition and subsoil aluminium concentration. Although no suitable predictor of N deposition has

been identified, species richness may provide a suitable indicator of ecosystem health in response to N deposition.

7. Conclusions

Although there has been considerable research into the potential effect of N deposition on semi-natural habitats, there has previously been scant attention to whether these changes are actually occurring. The main aim of this project was to determine whether a relationship existed between rates of ambient nitrogen deposition in the UK and a) vegetation community structure, b) soil and vegetation mineral content and c) soil processes. This aim was achieved by examining 68 grassland sites along the UK gradient of inorganic N deposition. Modelled N deposition at the sites visited ranged from 6 – 36 kg N ha⁻¹ yr⁻¹. At every site, vegetation was surveyed, and topsoil and subsoil samples and plant tissue samples were collected. U4 (*Festuca ovina*-*Agrostis capillaris*-*Galium saxatile*) grassland was identified as a community likely to be sensitive to N deposition. Its poorly buffered soils make it sensitive to acidification and the low Ellenberg scores for nutrient status (N) suggested sensitivity to eutrophication.

7.1 Vegetation

- Species richness of U4 grasslands showed a linear negative trend with N deposition (figure 3.6), indicating that for every 2.5 kg ha⁻¹ yr⁻¹ of inorganic N currently deposited on an acid grassland, a mean of 1 additional species is excluded from a randomly placed 4m² quadrat. I hypothesise that this reflects the long-term influence of chronically elevated N deposition since at least the mid-twentieth century.

- The equation derived from this study predicts an average reduction in species richness (from a projected pristine condition of $5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) of 5.2% at the mean N deposition rate for eastern US and 23% at the mean rate for central Europe (equation 3.1).
- Forbs accounted for the majority of the decline in species richness, declining in both cover and richness with increasing N deposition. Grasses showed a slight increase in cover, but a slight decrease in richness, indicating increased dominance of a few species (figures 3.10A and B).
- Species diversity (using the Shannon diversity index) also showed a linear negative trend with N deposition (figure 3.13).
- Canonical correspondence analysis (CCA) showed that N deposition has an important influence over vegetation species composition, explaining a large amount of variation between sites (figure 3.4).
- Altitude, climatic variables and S deposition were all correlated with N deposition in the CCA ordination (figure 3.3).
- The abundance of *Vaccinium myrtillus*, *Hypnum cupressiforme* and *Carex panicea* showed a positive correlation with N deposition (figure 3.5). Abundance of *Nardus stricta* and *Dicranum scoparium* showed a weaker positive correlation with N deposition.
- *Plantago lanceolata*, *Hylocomium splendens*, *Euphrasia officinalis*, *Campanula rotundifolia*, *Calluna vulgaris* and *Lotus corniculatus* all show lower abundance at high N deposition (figure 3.5).

7.2 Soils

- Both topsoil and subsoil pH were negatively correlated to N deposition (both $r^2=0.33$, $p<0.01$, equations 4.6 and 4.8). The inclusion of S deposition improved the regression for topsoil pH ($r^2=0.65$, $p<0.001$, equation 4.7) but not subsoil pH.
- A number of metals were found to be increasingly available in the soil with increasing N deposition and decreasing pH *i.e.* aluminium and lead. For aluminium and lead, it was possible to identify pH thresholds below which there was increased mobilisation (figure 4.9).
- Some soil extractable metals, especially base cations, showed a negative correlation with N deposition and a negative correlation with pH. These included calcium, lithium, sodium and magnesium (table 4.1).
- There was no significant relationship between total soil N and total inorganic N deposition (figure 4.4). However, there was a weak relationship between the C:N ratio of the A horizon and N deposition (figure 4.5).
- KCl-extractable soil nitrate did not show a significant relationship with total inorganic N deposition (figure 4.6). KCl-extractable ammonia was much more closely related to total inorganic N deposition and NH_x deposition ($r^2=0.39$, $p<0.01$, $r^2=0.34$ $p<0.01$ respectively, figure 4.7).
- The results confirm observations made in experimental N manipulations and demonstrate that they may actually be occurring in a semi-natural system in the UK.
- NH_4^+ mineralization showed no significant trend in relation to N deposition, although within site-variation was large (figure 4.11).

7.3 Plant Tissues

- There was no correlation between total inorganic N deposition and above-ground tissue N and N:P ratio for any of the species tested (*Agrostis capillaris*, *Galium saxatile* and *Rhytidiadelphus squarrosus*; figures 5.1, 5.3 and 5.4).
- A weak relationship between tissue N and soil C:N ratio (figure 5.2) suggests that relationships between plant tissue N and N deposition identified in other studies may be more indirect, conveyed by the soils.
- Plant tissue N:P ratios do not give a clear indication of nutrient limitation in the present study (figure 5.6).

7.4 Plant-soil interactions

- N deposition (negative relationship), soil calcium concentration (+), altitude (-) and soil lithium concentration (+) together explain 77% of the variation in plant species richness in UK acid grasslands (equation 6.1).
- Species composition can also be related to pH (-), soil moisture deficit (+), S deposition (+) and soil aluminium concentration (+).
- Plant species richness may provide an indicator of ecosystem health in response to N deposition.

7.5 Suggestions for further study

The next stage for this project should be validation of the model that has been developed for species richness in relation to N deposition. This would require a further survey of new U4 grassland sites. It would also be very interesting to examine relationships between N deposition and species richness in different communities

(especially MG5, identified as another potentially sensitive community in chapter 2), and grasslands fitting the U4 classification from further afield, particularly high deposition areas of Europe. It would also be very interesting to repeat this survey in the future to determine the effect of changes in N deposition and possible recovery or deterioration.

This project has also highlighted areas that would benefit from more in-depth study. It would be very interesting to look at the relationships between N deposition and the plant uptake of N in the soil and how this might be influencing plant species richness and composition. It would also be useful to investigate the availability of different forms of soil P in relation to N deposition (as identified in section 4.4.4).

Further work is also needed to establish nutrient limitation at the sites and potential changes from N limitation to P limitation with increasing N deposition. Changes in vegetation productivity in relation to N deposition could be investigated by harvesting biomass from grazing exclosures.

8. Acknowledgements

Firstly I would like to thank my project supervisors Nancy Dise and David Gowing at The Open University and Owen Mountford at the Centre for Ecology and Hydrology, Monks Wood for all their help and support throughout the project, for conceiving the initial idea and for fighting for funding.

I would like to thank the Fergusson Fund, the Department of Earth Science and the Open University Research Development Fund for funding the project and studentship and NERC for acting as a CASE partner. I would also like to thank the NERC GANE programme for allowing the project to be associated with them.

I am very grateful to Graham Howell and Meaghan Jenkins for assistance and advice in the laboratory. I am also grateful to the following for their help as field assistants: Mark Stevens, David Bilbie (MSc student, Wolverhampton University), Jason Harvey, April Clarke, Lee Bates, Alma Rodhe, Owen Mountford, Nancy Dise, David Gowing, Jessica Homer and Severine Machefert. I would also like to thank all the landowners that allowed access to their land especially the Ministry of Defence. I am extremely grateful to staff at English Nature, Countryside Council for Wales and Scottish Natural Heritage who assisted with identifying sites and access permission.

I am grateful to Ruth Swetnam for assistance with GIS, Roland Bol for assistance with nitrogen isotope analysis, Angela Coe for use of the LECO and Dan Croucher and John Watson for assistance with running it, Nick Rogers for use of the ICP-MS and Fatima Mokadem for assistance with running the samples. I would also like to

thank the following for advice, support and assistance during the course of my PhD:
My family (especially Gayna Stevens), Mark Brandon, Bridgett Emmett, Jonathan Silvertown, Mike Dodd, Louis de la Foret, Sheila Anderson, Andrew Sugden, Mark Sephton, Mark Gardener, Yoseph Araya and Andrew Hobson.

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Appendix 1 – Grassland Communities

Descriptions of grassland communities (taken from Rodwell, 1992).

MG1 *Arrhenatherum elatius* grassland

MG1 is a community dominated by coarse-leaved grasses, especially *Arrhenatherum elatius*, *Dactylis glomerata* and *Holcus lanatus*. Large umbellifers and tall herbs can be locally dominant. Other finer-leaved grasses and small forbs form a second layer of vegetation below. Vegetation is generally tall and species poor.

MG1 is a very common grassland of circumneutral soils, typical of roadside verges, neglected farmland and post-industrial sites. It is typically an ungrazed grassland maintained by mowing. Without management it undergoes succession initially forming large tussocks and as organic matter accumulates eventually changing to scrub then woodland.

MG1 is virtually ubiquitous throughout the UK.

MG5 *Cynosurus cristatus*-*Centaurea nigra* grassland

MG5 is a species rich grassland typified by a high forb diversity. Species including *Festuca rubra*, *Cynosurus cristatus*, *Agrostis capillaris*, *Lotus corniculatus*, *Plantago lanceolata* and *Trifolium repens* are common but do not consistently dominate. It is a community that is known to contain a number of species of limited distribution. The vegetation can vary from being short and quite sparse to a lush tall growth.

Management is typically that of traditional grazed hay meadows with a cut for hay mid-summer followed by aftermath grazing until early spring. Some MG5 grasslands have been under this form of management for a considerable time and consequently have a very high diversity and form an attractive sward. Patchiness within the community is frequently related to soil pH or moisture. Insufficient management can lead to the formation of MG1 grasslands. It is a community commonly affected and degraded by agricultural intensification.

MG5 grasslands occur in the lowlands throughout the UK, especially in the Midlands.

MG6 *Lolium perenne*-*Cynosurus cristatus* grassland

MG6 grassland typically has a low species diversity dominated by *Lolium perenne* with varying amounts of *Cynosurus cristatus*. *Agrostis capillaris* and *Holcus lanatus* are common. There are few forbs although *Trifolium repens* is usually very abundant. *Senecio jacobaea* and *Cirsium arvense* are common, causing problems for the management of these grasslands.

MG6 is the dominant pastureland in the UK. It is also widespread as a recreational sward. Management is by grazing, cutting or mowing and will commonly be for productivity in agricultural systems leading to the very low diversity typical of this grassland. The nature of the grassland generally results in little variation. Insufficient grazing leads to an increase in coarser species leading to MG1 grassland and eventually scrub formation.

The community is virtually ubiquitous in the British lowlands, especially common in the major dairy farming areas.

MG10 *Holcus lanatus*-*Juncus effusus* rush-pasture

The sward of this community is generally dominated by stands of *Juncus effusus* up to 80cm tall with a species poor grassy understorey dominated by *Holcus lanatus* and *Agrostis stolonifera*. *Ranunculus acris* and *R. repens* can be abundant and other common forbs such as *Rumex acetosa* and *Trifolium repens* can occur.

The community is common on a wide variety of soils throughout the British lowlands and upland fringes. It found in areas of consistently high soil moisture and is generally maintained by grazing. The *Juncus* can survive high intensity grazing as it is resistant to trampling and is moderately unpalatable. It most commonly occurs in a mosaic with other grassland or wetland habitats.

This community is common throughout the British lowlands.

MG11 *Festuca rubra*-*Agrostis stolonifera*-*Potentilla anserina* grassland

MG11 is a variable community generally typified by species poor sward in which *Agrostis stolonifera* and *Festuca rubra* are the dominant grasses. *Potentilla anserina* cover can be very variable becoming dominant in parts. With the exception of *Trifolium repens* there are few other forbs.

This is a lowland vegetation type typical of free-draining circumneutral soils that are frequently inundated. It is most common near sea level or as fragments along drainage ditches or in damp woodland rides. The community is often grazed by sheep and cattle and can be subject to agricultural improvement. This can lead to patches of nitrophilous species such as *Urtica dioica*.

The community is most common in lowland river valleys, particularly in the Midlands.

U4 *Festuca ovina*-*Agrostis capillaris*-*Galium saxatile* grassland

A mix of grasses including *Festuca ovina* or *F. rubra*, *Agrostis capillaris* and *Anthoxanthum odoratum* commonly dominates U4 grassland. A number of other grasses occur in the short sometimes tussocky sward but do not become dominant, these include *Danthonia decumbens*, *Holcus lanatus*, *Molinia caerulea* and *Nardus stricta*. The forb flora is commonly species poor although this is not always the case. *Galium saxatile* and *Potentilla erecta* are constant accompanied by a range of other acid tolerant species. Bryophytes occur commonly although are not usually very abundant. A number of woody species occur including *Calluna vulgaris* and *Vaccinium myrtillus*.

This community is the most common extensive pastureland in sub-montane areas of Britain. It is of great agricultural importance for rough grazing. It is grazed predominantly by sheep, which prevent the regression to domination by woody

vegetation. It is frequently agriculturally improved and can be affected by *Pteridium aquilinum* invasion.

U4 is virtually ubiquitous throughout the sub-montane zone.

Appendix 2 – Site names and locations

Site names and grid references (British national grid).

Site Number	Site Name	Map Grid Reference
1	Braemore Estate	NH277763
2	Ben Klibreck	NC542294
3	Ardnamurchan estate	NM513643
4	Newtonmore	NN688997
5	Pitarrig Meadow	NN960598
6	Tynewydd	SN796736
7	Gamallt	SN775557
8	Carn Pembewllidog	SN732522
9	Mark Ash Wood	SU243074
10	Wilverly Plain	SU245015
11	Broomy Plain	SU201107
12	Bury Castle	SS917473
13	Appleby Fells	NY729225
14	Finglandrigg Woods South	NY274565
15	Finglandrigg Woods North	NY270567
16	Grey Mares Tail	NT185156
17	Carnbee Law	NO522068
18	Moorfoot Hills	NT346444
19	Hardgate Head	NT129497
20	Tweedsmuir	NT134288
21	Tred-rhiw-draen	SN898664
22	Sennybridge	SN970467
23	Dolobran	SH846176
24	Brynbedu	SS957920
25	Mynydd Hiraethog	SH977584
26	Croxton Park	SK821276
27	Foreland Point	SS751507
28	Valley of the Rocks	SS714498
29	Sharp Tor	SX684733
30	Sinks Valley	TM227462
31	Madge Hill Farm	SE180669
32	Great Dunn Fells	NY716309
33	Hathersage Moor	SK261824
34	Winsford Hill	SS894326
35	Molland Common	SS822315
36	Stainton Moor	SE075934
37	Burbage Moor	SK265812
38	Blacka Moor	SK283799
39	Withypool Common	SS814364
40	Craigmead	NO129061
41	Glamorgan Uplands	ST101918
42	Rough Tor	SX144811
43	Merryton Low	SK046612
44	High Fields	SK036593

Site names and grid references (continued)

Site Number	Site Name	Map Grid Reference
45	Harpersend	SK012629
46	Roach End	SJ994644
47	Spring Head	SK013667
48	Hob Hay	SK047603
49	Ramshaw Rocks	SK020628
50	Five Clouds	SK002625
51	Roach End	SJ996644
52	Adders Green	SK020652
53	Upper Hay	SK047605
54	Under Hill	SK015669
55	Hazel Barrow	SK015637
56	Doxy Pool	SK007623
57	Great Hucklow	SK188784
58	Snailsdon	SE131043
59	Hoyles Farm	SK232893
60	Beecraigs	NS997741
61	Lundy North End	SS132465
62	Lundy Middle Field	SS135463
63	Lundy South	SS132443
64	Ardmaenach	NM454327
65	Cuillins	NG411241
66	Bein na h-Uamha	NM769537
67	Glen Nevis	NN136704
68	Tulach Hill	NN882633

Appendix 3 - Residuals

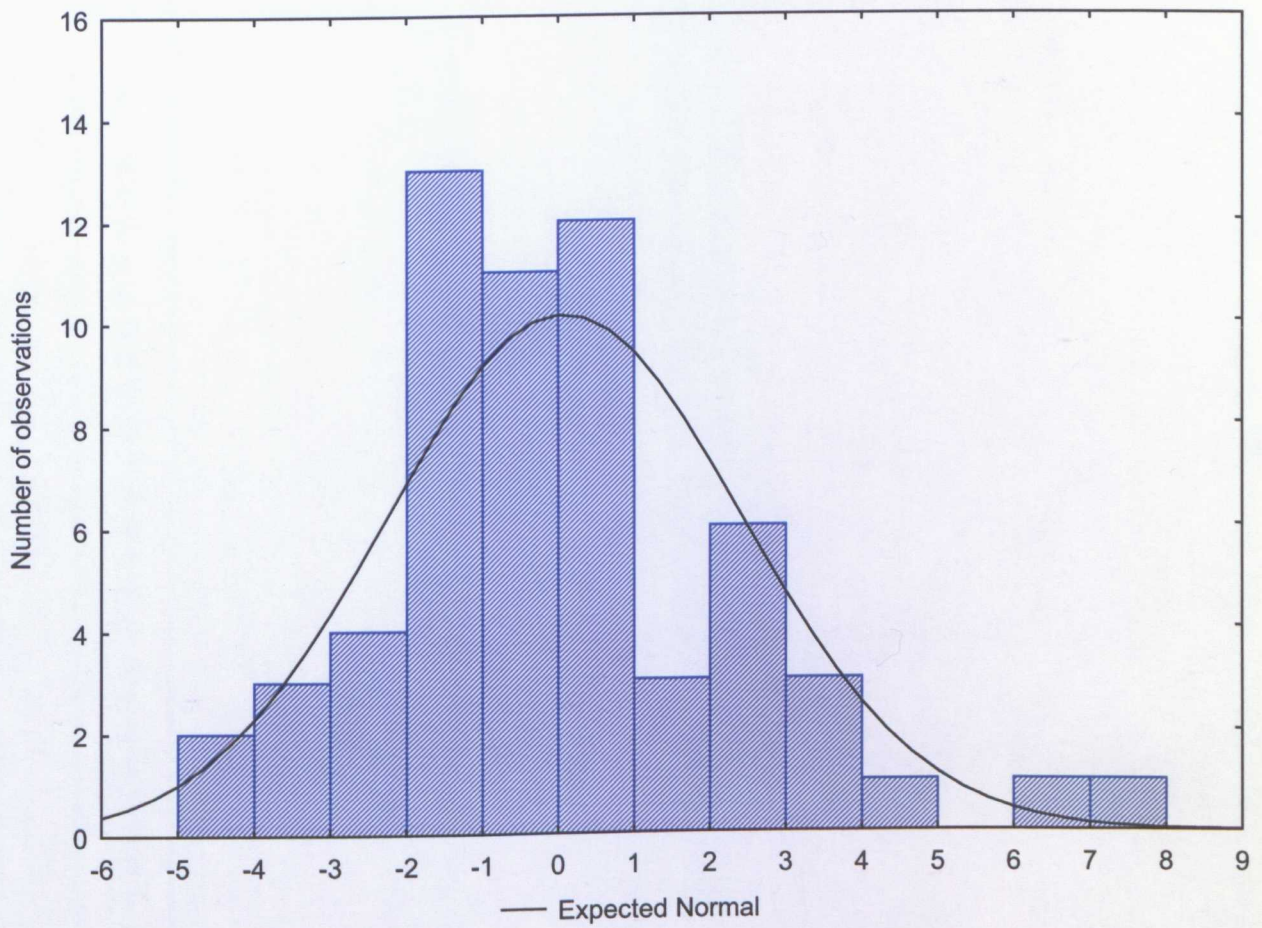


Figure A3.1 Distribution of raw residuals.

Appendix 4 – Species richness

Mean Species richness of 5 quadrats for each site.

Site	Species richness
1	20.8
2	18
3	25.4
4	19.4
5	20
6	10.8
7	13.6
8	14
9	15.4
10	15.8
11	21.6
12	13.8
13	17.6
14	15.4
15	15.2
16	15.2
17	14
18	13
19	14
20	16
21	10.4
22	14
23	11
24	7.2
25	16.2
26	11.6
27	9
28	11.6
29	15.4
30	9.4
31	12.2
32	14.8
33	9.4
34	11.6
35	11.6
36	9
37	14.8
38	11
39	12.4
40	20.6
41	10
42	10.4
43	8.8
44	10.2
45	9.8
46	9.2
47	12.2
48	9.4
49	11

Site	Species richness
50	8.6
51	8.2
52	12.2
53	8
54	12.2
55	11
56	9.4
57	14.4
58	8.8
59	13.4
60	13.8
61	21
62	17.2
63	16
64	27
65	27.6
66	20
67	20.6
68	26

Appendix 5 – Ellenberg scores

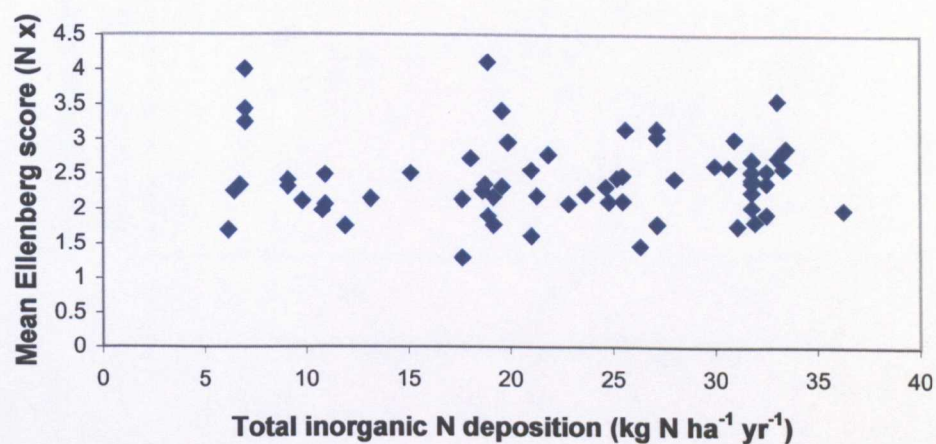


Figure A5.1 Ellenberg scores (Hill *et al.*, 1999) for N (nutrient status) versus total inorganic N deposition. Low scores represent indicators of low nutrient status. Scores for species originally (Ellenberg, 1979) denoted by an x (broad amplitude) have been removed.

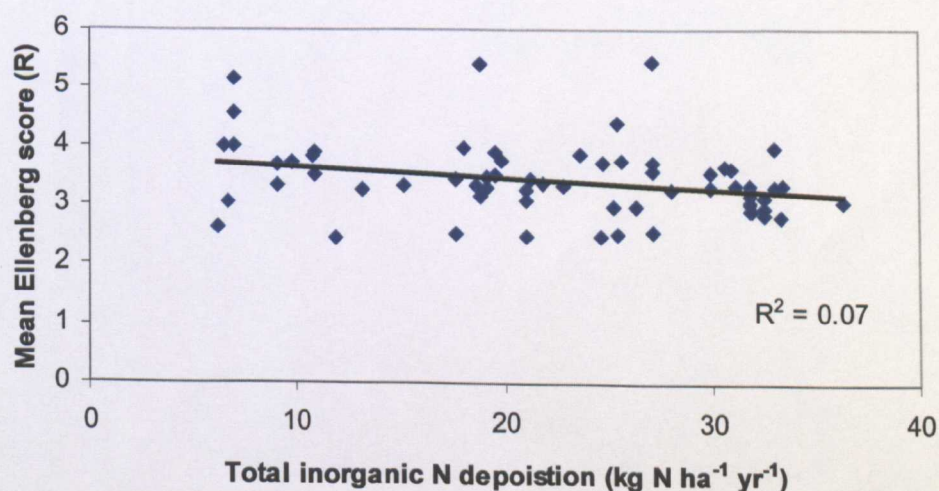


Figure A5.2 Ellenberg scores (Hill *et al.*, 1999) for R (reaction) versus total inorganic N deposition. A low score represents indicators of acid environments.

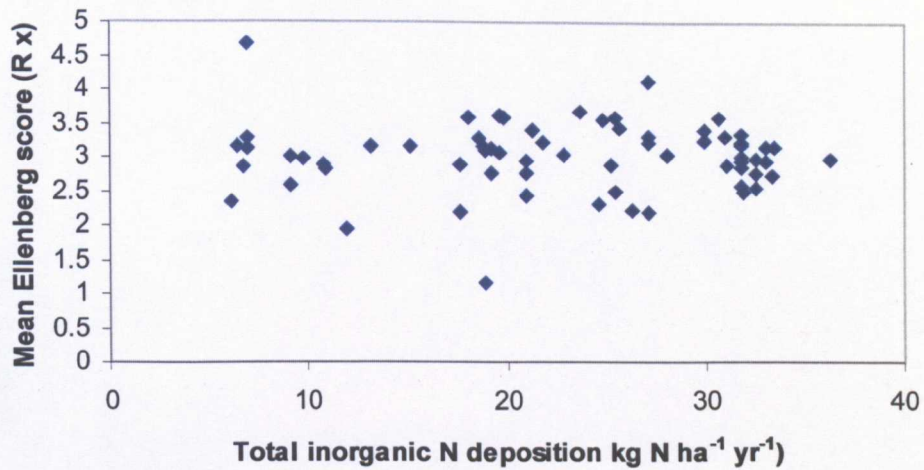


Figure A5.3 Ellenberg scores (Hill *et al.*, 1999) for R (reaction) versus total inorganic N deposition. Scores for species originally (Ellenberg, 1979) denoted by an x (broad amplitude) have been removed.

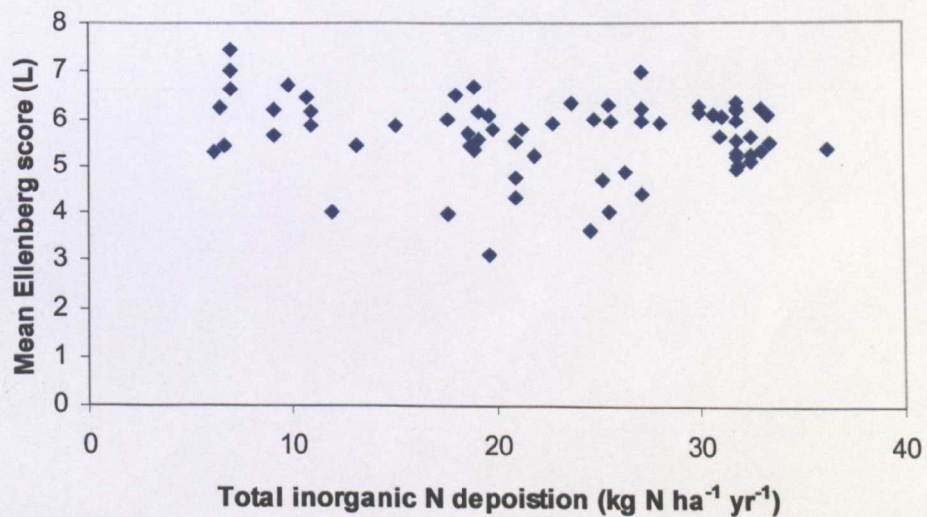


Figure A5.4 Ellenberg scores (Hill *et al.*, 1999) for L (light) versus total inorganic N deposition. A low score represents indicators of deep shade.

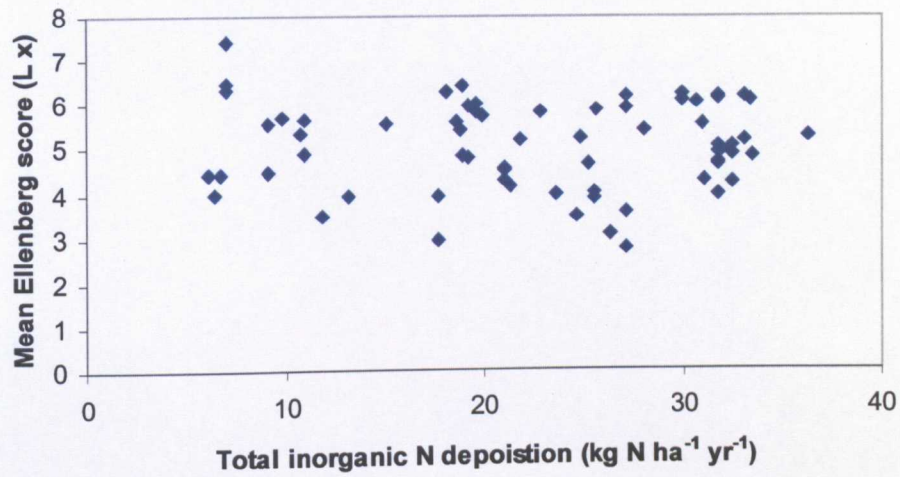


Figure A5.5 Ellenberg scores (Hill *et al.*, 1999) for L (light) versus total inorganic N deposition. Scores for species originally (Ellenberg, 1979) denoted by an x (broad amplitude) have been removed.

Appendix 6 – Topsoil and subsoil metal correlation matrix

Correlation matrix showing correlations between topsoil and subsoil metal concentrations. Significant correlations are show in red (p<0.05).

	Topsoil															
	Li	Na	Mg	Al	Si	Ca	Cr	Mn	Fe	Ni	Cu	Zn	As	Ba	Pb	Al:Ca
Subsoil	Li	0.33	-0.01	-0.09	-0.10	0.00	0.42	-0.09	0.00	0.32	0.08	0.56	-0.23	0.49	-0.17	-0.01
	Na	0.28	0.54	0.56	-0.19	0.33	0.08	0.45	-0.10	0.45	0.08	0.30	-0.34	0.12	-0.31	-0.15
	Mg	0.13	0.20	0.43	-0.23	0.23	0.23	0.11	-0.15	0.12	-0.12	0.03	-0.22	0.00	-0.22	-0.23
	Al	-0.27	-0.28	-0.22	0.57	-0.15	-0.13	-0.19	0.53	-0.20	0.08	-0.29	0.29	-0.13	0.43	0.48
	Si	-0.07	-0.20	0.19	0.08	0.17	0.01	0.08	-0.04	0.07	-0.02	0.13	-0.32	0.11	-0.11	0.13
	Ca	0.04	0.03	0.31	-0.20	0.15	0.43	-0.03	-0.26	-0.02	-0.17	-0.06	-0.23	0.06	-0.16	-0.31
	Cr	0.07	-0.03	0.08	-0.04	0.09	-0.04	0.19	-0.13	0.02	0.14	-0.09	0.24	0.19	-0.14	-0.10
	Mn	-0.02	-0.06	-0.10	0.26	-0.09	0.31	-0.20	0.63	-0.12	-0.15	0.01	-0.23	0.07	0.13	-0.05
	Fe	0.03	-0.11	-0.02	0.00	0.08	-0.10	0.19	-0.24	0.18	0.14	-0.05	0.27	-0.04	0.19	0.04
	Ni	0.03	-0.03	0.09	-0.06	0.07	-0.04	0.15	-0.13	0.00	0.11	-0.12	0.20	-0.18	0.16	-0.14
	Cu	0.17	-0.15	-0.18	0.07	0.17	-0.16	0.30	-0.16	0.21	0.21	0.32	0.44	-0.20	0.42	0.17
	Zn	0.29	-0.10	-0.09	-0.09	0.05	-0.04	0.51	-0.11	0.02	0.40	0.09	0.70	-0.30	0.70	-0.06
	As	-0.34	-0.19	-0.11	0.05	-0.15	-0.06	-0.19	-0.18	-0.09	-0.11	-0.09	-0.31	0.19	-0.17	0.24
	Ba	0.25	-0.12	-0.02	-0.10	0.08	0.05	0.48	-0.12	-0.03	0.39	0.06	0.62	-0.42	0.80	-0.08
	Pb	-0.28	-0.32	-0.27	0.20	-0.07	-0.06	-0.18	-0.23	0.24	-0.16	0.02	-0.25	0.12	0.04	0.62
Al:Ca	-0.14	-0.11	-0.15	0.27	-0.21	-0.35	-0.13	-0.04	0.52	-0.16	-0.01	-0.22	0.31	-0.28	0.10	0.46